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MYCORRHIZAL STUDIES REGARDING THE RECLAMATION OF OIL SAND TAILINGS: PRODUCTION AND OUTPLANTING OF JACK PINE SEEDLINGS AND AMOUNTS OF VA- AND ECTOMYCORRHIZAL INOCULUM IN STOCKPILED PEAT

edited by

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EXECUTIVE SUMMARY

The major objectives of the ectomycorrhizal studies were to produce mycorrhizal jack pine seedlings in the greenhouse and to initiate a field study on the Syncrude dyke on the effect of different ectomycorrhizal fungi on the growth of jack pine. In addition, a simple inoculation technique which would allow inoculations to be performed under operational conditions was to be tested as well as the effect of different fertilizer levels on seedling growth and ectomycorrhizal development. These studies would then indicate the feasibility and limitations of inoculating with specific fungi and give information on the field performance of both fungi and pine seedlings in an actual reclamation situation.

Under current practices, ectomycorrhizal tree species may acquire their symbionts while in the nursery, from the reconstructed soil they are planted in, or through air-borne sources after outplanting. VA mycorrhizal shrubs are dependent upon the same sources whereas plants seeded directly are totally dependent upon the reconstructed soil for inoculum. As a first step in evaluating the importance of these inoculum sources, an objective of the current study was to determine the relative amount of both ectomycorrhizal and VA mycorrhizal inoculum in undisturbed muskeg and in stockpiled muskeg. In that fungi differ in their effects on plant growth, identification of the symbionts was also attempted.

The specific studies reported here are (1) the production of ectomycorrhizal jack pine seedlings under experimental conditions, (2) the outplanting of these seedlings on the Syncrude dyke and the first season's results, (3) the testing of near-operational fertilizer regimes on mycorrhizal development in the greenhouse, (4) the use of a simple slurry technique for inoculating seedlings, (5) the use of a bioassay technique to determine the effects of stockpiling muskeg peat on ectomycorrhizal and VA mycorrhizal inoculum, and (6) a comparison of ectomycorrhizal development in the field and in the greenhouse to test the validity of the bioassay technique.

The jack pine seedlings produced in the greenhouse were

below the normal nursery target size due to the low fertilizer regime. However, the use of low nutrient levels permitted mycorrhizal development by 9 of the 12 fungi tested. The most aggressive fungi (those producing the highest levels of short root infection) were Thelephora terrestris, Laccaria proxima, Hebeloma sp. and E-strain. All of these are known as "weedy" species and are commonly found in nurseries. A lower degree of infection was achieved with Cenococcum geophilum, Pisolithus tinctorius, Astraeus hygrometricus, Lactarius paradoxus and Sphaerosporella brunnea. Amphinema byssoides, Hydnum imbricatum and Tricholoma flavovirens failed to form any mycorrhizae.

After one season in the field, T. terrestris, L. proxima, Hebeloma sp. and E-strain had all readily infected the new roots that extended into the reconstructed soil. The other fungi were poor colonizers of jack pine roots in the field. Competition from indigenous fungi was not a factor in the degree of success as only 4% of the short roots were infected by indigenous species. Growth of jack pine was not significantly affected by the presence of mycorrhizae during the first growing season.

It was necessary to produce larger seedlings if inoculations were to have any practical value. In a fertilization experiment, it was found that E-strain fungi and Laccaria proxima would aggressively infect jack pine roots at approximately one-half the operational fertilizer rate. The seedling size was acceptable for normal outplanting. Pisolithus tinctorius and Sphaerosporella brunnea were more sensitive to high fertilizer rates than the former two fungi.

The standard inoculation procedure requires that the inoculum be grown in a solid carrier several months prior to seeding the containers and that inoculation takes place as the growing medium is added to the containers. However, experimental evidence presented here shows it is also possible to use an easily prepared mycelial slurry which can be injected into the individual cells after the seedlings are two months old. The slurry infection technique proved to be just as effective as the solid carrier technique for aggressive species. The technique offers considerable time-saving advantages as well as simplifying experimental inoculations in operational nurseries.

In the process of mining, the muskeg peat is stripped off and stockpiled until it is required for the reclamation of the tailings sand. The greenhouse bioassay used here clearly shows that the amount of ectomycorrhizal inoculum is reduced by stockpiling. It reduced infection levels of the jack pine test seedlings, resulted in fewer ectomycorrhizal seedlings and a strong reduction in the number of symbiont species present. The bioassay technique for ectomycorrhizal inoculum was effective at detecting viable mycorrhizal fungi but had limited quantitative predictive value for field situations. VA mycorrhizal inoculum was very rare in both undisturbed peat and peat stockpiled for 8 months. This was due to the rare occurrence of compatible hosts in muskeg plant communities. However, when stockpiles were seeded with grasses and legumes, there was a slow build-up of VA inoculum.

From these studies it can be recommended that:

1) Monitoring of the ectomycorrhizal inoculation study on the Syncrude dyke be continued;

2) Strong efforts should be made to improve inoculation techniques, especially with regard to those fungi which consistently fail to survive in the growing medium. This will involve basic studies of survival mechanisms and microfloral interactions;

3) A wide range of potential symbionts should be tested for their sensitivity to a range of fertilizer regimes in the greenhouse;

4) In that VA mycorrhizal inoculum in peat is sparce, the mycorrhizal dependency of all VA hosts used in the reclamation of tailings sand should be determined;

5) The mycorrhizal condition of VA hosts planted on the Syncrude dyke should be evaluated to determine if the absence of inoculum is potentially limiting reclamation progress;

6) The development of indigenous ectomycorrhizal populations in the outplanting study should be followed and the effectiveness of these fungi evaluated;

7) The other major input of ectomycorrhizal inoculum should be evaluated. This would involve determining the species of fungi present on nursery stock, the degree of colonization of the roots, and the persistence of these fungi following outplanting; and

8) The use of mineral topsoil as a source of VA mycorrhizal inoculum should be evaluated. VA mycorrhizal hosts are common in aspen stands and the addition of a small amount of soil from these stands to reconstructed soils may be sufficient to establish VA mycorrhizal systems.

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1. GENERAL INTRODUCTION

Based on quantitative observations of mycorrhizae developing on white spruce and jack pine grown in amended spoils and on the occurrence of fruit bodies of mycorrhizal fungi in the field, a preliminary group of fungi has been selected for on site performance testing. Not only does this include the performance of the host with regards to growth and survival but also the performance of the fungal symbiont in pure culture, the ability of the symbiont to infect significant portions of the host root system in the greenhouse and finally, the ability of the symbiont to persist and infect new roots in the field. In the case of the latter two factors, the fungus must be able to colonize and infect short roots under moderately high nutrient regimes, in the greenhouse or nursery and also to perform symbiotically in the field under low nutrient conditions.

Information regarding fertilizer tolerance limits of each symbiont under greenhouse rearing conditions is urgently required so that reasonably sized seedlings infected with specific symbionts can be produced for field testing. Further, it is critical to develop inoculation techniques that will allow testing of the numerous and promising symbionts which to date have defied successful manipulation and artificial inoculation.

Although mycorrhizal associations can be established on planting stock with reliable replication, further problems can be anticipated once the seedlings are outplanted. Performance data of the host cannot be correctly interpreted without knowledge of the indigenous symbionts, i.e. abundance, identity, aggressiveness, effectiveness and competitiveness with introduced symbionts. Sources and amounts of inoculum of both VA- and ectomycorrhizal fungi must be defined in the event that later enhancement or control is desired. Once factors concerning seedling production, indigenous mycorrhizal populations and the development and persistence of associations have been detailed, the possibilities of using mycorrhizal management as a tool in revegetation schemes can be properly tested.

2. PRODUCTION OF ECTOMYCORRHIZAE ON CONTAINER-GROWN JACK PINE SEEDLINGS

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2.1 ABSTRACT

Jack pine seedlings were grown for 20 wk in a peat-vermiculite medium inoculated with solid carrier mycelial inoculum. The low fertilizer levels used resulted in seedlings below standard for outplanting but permitted mycorrhizal development by 9 of the 12 fungi tested. Greater than 90% of the short roots were infected when seedlings were inoculated with *Thelephora terrestris*, *Laccaria proxima*, *Hebeloma* sp. or E-strain. About half the short roots were infected when *Cenococcum geophilum*, *Pisolithus tinctorius* and *Astraeus hygrometricus* were used. Thirty two and 17% of the short roots were infected by *Lactarius paradoxus* and *Sphaerosporella brunnea*, respectively. Inoculation with *Amphinema byssoides*, *Hydnum imbricatum* and *Tricholoma flavovirens* failed.

2.2 INTRODUCTION

Although extensive greenhouse and field testing of the efficiency of ectomycorrhizal fungi has occurred in the past decade, it has been largely restricted to a few fungal species in south temperate regions (e.g. Marx et al., 1982). The few studies that have been done in the northern U.S. and Canada have utilized some of the same fungi, especially *Pisolithus tinctorius*, used in southern locations (Navratil et al., 1981; Riffle and Tinus, 1982). With the exception of *Pisolithus* and *Thelephora terrestris*, the establishment of other symbionts on container-grown seedlings in northern nurseries has proven to be difficult (Riffle and Tinus, 1982) or very erratic (Shaw et al., 1981). In that *Pisolithus* has rarely been found in Canada (Malloch and Kuja, 1979), it is likely that other species of fungi may be better adapted to the harsh Canadian climate and will benefit plant growth to a greater degree than that afforded by *Pisolithus*. Navritil et al. (1981) demonstrated that inoculation of jack pine with *Pisolithus* resulted in increased growth but suggested that symbionts native to the boreal forest should be selected and tested in a similar manner.

The objective of this study was to select symbionts covering a wide taxonomic spectrum that occurred in a range of environmental conditions and to establish mycorrhizae with these fungi on container-grown jack pine. The mycorrhizal plants were subsequently outplanted in northern Alberta to determine if the symbionts persisted on the root system and how each species affected plant growth and survival. Outplanting results are reported in Part 3.

2.3 MATERIALS AND METHODS

The fungi used in the inoculation trial were all from Alberta with the exception of *Pisolithus* (Table 1). Most of the fungi were cultured from basidiomes from two northern Alberta sites, the Richardson Fire Tower and Mildred Lake. Voucher specimens and cultures are on deposit at the Biosystematics Research Institute, Ottawa.

Inoculum was prepared in respiration tubes (Visser, unpubl.

Table 1. Mycorrhizal fungi tested in the greenhouse inoculation trial.

Species and isolate number	Source and date of isolation	Habit and associates
Astraeus hygrometricus (Pers.) Morgan, RMD 2186	Richardson Fire Tower, Aug. 11 1976; basidiome	Jack pine seedlings, old burn
Amphinema byssoides (Fr.) J. Erikss., R-7	Ribbon Creek mine spoil, Oct. 3, 1975; mycorrhiza	Outplanted white spruce seedling
Cenococcum geophilum Fr., R-26	Richardson Fire Tower, Oct. 8 1975; sclerotium	Mature jack pine - lichen woodland
E-strain (sensu Mikola), R-947	Calgary, fertilized subalpine spoil; mycorrhiza	White spruce seedling
Hebeloma sp., RMD 2657	Mildred Lake; Sept. 21, 1977; basidiome	Cutline, bearberry and jack pine
Hydnum imbricatum L. ex Fr., RMD 2737	Brown Lowery Provincial Park, July 22, 1978; basidiome	Mature white spruce
Laccaria proxima Boudier, RMD 2661	Mildred Lake, Sept. 21, 1977; basidiome	Cutline, bearberry and jack pine
Lactarius paradoxus Beardslee & Burlingham, RMD 2454	Richardson Fire Tower, Sept. 25, 1976; basidiome	Roadcut by jack pine stand
Pisolithus tinctorius (Pers.) Coker & Couch, Marx 185	Fort Lawson, Oklahoma; Dec. 1975; mycorrhiza	Loblolly pine nursery
Sphaerosporella brunnea (Alb. & Schw. ex Fr.) Svrcek & Kubicka RMD 1864	Calgary, greenhouse; Sept. 25, 1978; mycorrhiza	Container-grown jack

(cont'd)

Table 1 (continued).

Species and isolate number	Source and date of isolation	Habit and associates
Thelephora terrestris Ehrhart ex Fr. RMD 3022	Mildred Lake, Sept. 16, 1980; basidiome	Cutline, jack pine seedlings
Tricholoma flavovirens (Pers. ex Fr.) Lundell RMD 3043	Mildred Lake, Sept. 16, 1980; basidiome	Cutline, jack pine seedlings

data) containing a 1:15 (v/v) mixture of peat and vermiculite moistened with modified Melin-Norkrans solution (MMN) (Marx, 1969). Malt extract solution was substituted for MMN for Cenococcum. The tubes were autoclaved for 15 min and inoculated with a mycelial slurry prepared from colonies growing on MMN agar. The tubes were incubated at room temperature for up to 3 mo depending on growth rates and the CO_2 evolution periodically determined with a Wosthoff CO_2 analyzer to monitor the physiological condition of each fungus (Visser and Danielson, unpubl. data). The inoculum was removed from the tubes, a subsample plated on MMN agar to determine viability and to check for contamination, and the remainder washed in cold tap water.

The inoculum was mixed 1:9 with a 1:1 mixture of peat and vermiculite. The peat had been autoclaved and reinoculated with a soil suspension from a prairie site several months previously and was pH 5.5. Leach 65 cm^3 Cone-tainers (Ray Leach, Canby, Or.) were filled with the planting mixture and a single, surface sterilized, pregerminated jack pine seed was planted in each cell.

Photoperiod in the greenhouse was extended to 20 h with a minimum of 3.5 klux and a temperature ranging from 18 to 25°C. The fertilizer regime was as follows: week 1, a single application of 10:42:10 at 80 mg l^{-1}; weeks 2 to 4 twice weekly applications of 15:15:18 at 50 mg l^{-1}; weeks 5 to 15 twice weekly applications of 15:15:18 at 100 mg l^{-1}; weeks 16 to 20, thrice weekly applications of 15:15:18 at 200 mg l^{-1}. Tap water was used to mix the fertilizer solutions for the first 13 wk but when it was determined that the pH of the fertilizer solution was 6.7, deionized water was substituted with the result that the pH of the solution was 4.7. Due to the development of Fe chlorosis in some treatments, 56 mg l^{-1} of Sequestrene was added with the fertilizer after the seventh week. Containers containing Thelephora and Laccaria were moved to a separate greenhouse after 11 wk as fruit bodies were developing in the containers.

At the end of 20 wk, 10 replicate seedlings from each treatment were randomly selected and seedling growth and mycorrhizal development evaluated. Shoots were removed, heights measured, the number of branches counted and dried at 80°C and weighed. Roots were washed free of planting mixture, cut into 2 to 3 cm segments and infection rated on 300 randomly selected short roots per seedling using 12-25X magnification. Whole mounts of short roots were observed with a 40X brightfield objective to determine infection in doubtful instances. Roots were dried at 80°C and weighed; the weight of the counted sample allowed calculation of total short roots per seedling and number of short roots per unit root weight.

2.4 RESULTS

Time to reach peak activity as indicated by CO_2 evolution varied considerably among the various fungi (Fig. 1). As observed previously (Visser and Danielson, unpubl. data) *Sphaerosporella* grew very rapidly and CO_2 evolution decreased quickly. Those fungi which grew the slowest were *Cenococcum*, *Amphinema* and *Tricholoma flavovirens*. *Lactarius paradoxus* required the longest time to reach peak activity. When inoculation took place, all of the fungi were exhibiting low levels of activity.

Seedlings produced under the low nutrient regime were small (Table 2) and well below the standards set for the size of plantable seedlings by Carlson (1979). He determined that jack pine seedlings should total 1 g in dry weight and have a shoot:root ratio of 2. The low levels of fertilizer used in this experiment were designed to maximize mycorrhizal development at the cost of producing large seedlings. Fertilizer levels were changed twice during the 20 wk growth period in accordance with visual observations of the seedlings. After 6 wk, seedlings inoculated with E-strain, *Thelephora* and *Laccaria* showed strong Fe deficiency symptoms and Sequestrene was applied and corrected these symptoms. During the latter half of the growth period it was readily apparent that the seedlings inoculated with *Laccaria*, *Thelephora* and E-strain were smaller than seedlings in the other inoculation treatments. In that uniform sized seedlings were desired for outplanting, fertilizer levels were increased for all treatments. As a result, the final size of the seedlings were very similar at the end of the 20 wk period in the greenhouse. The larger size of the seedlings inoculated with *Amphinema* appears to be a spurious result as no

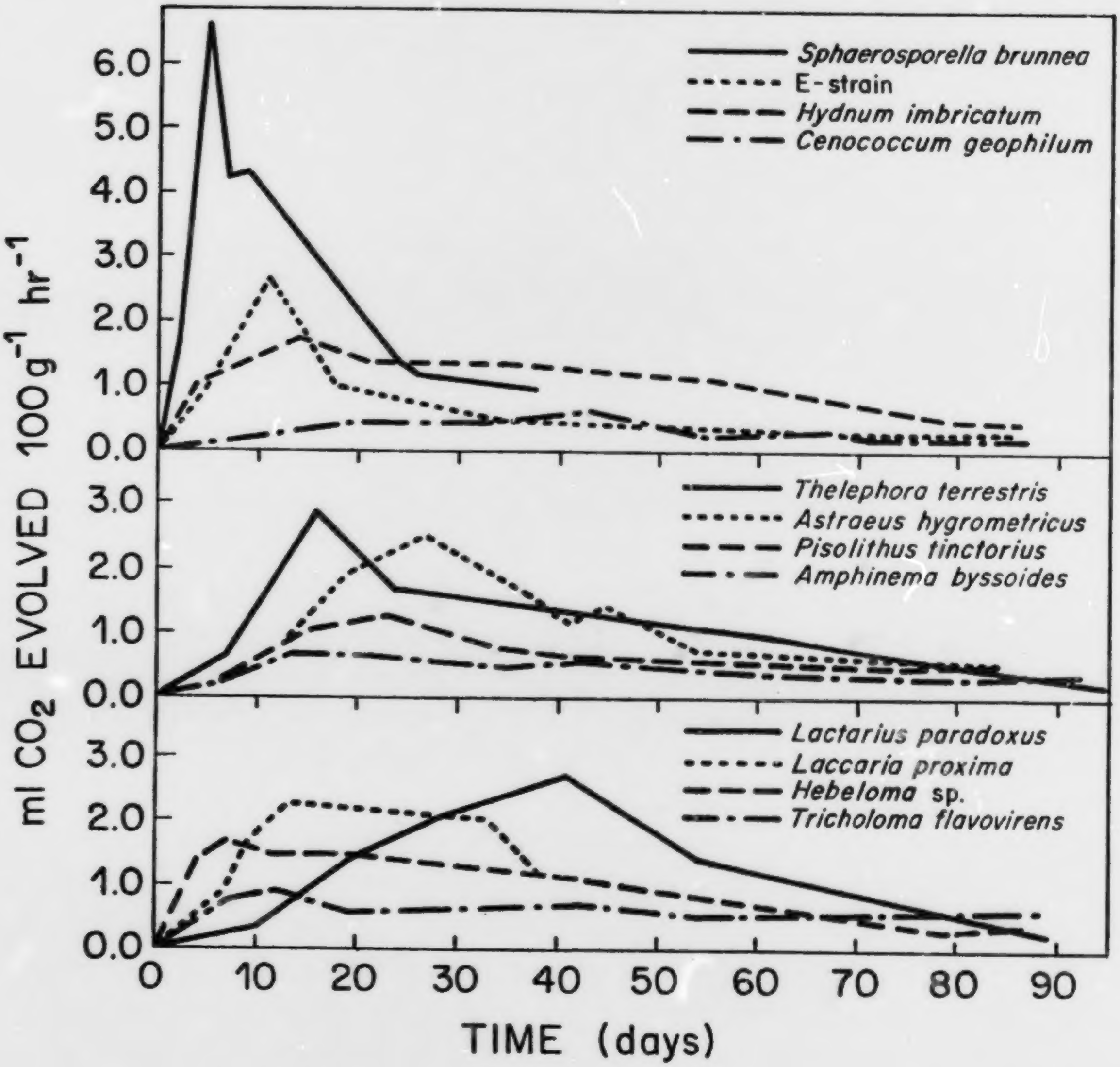

Figure 1. Respiration patterns of ectomycorrhizal inoculum in peat-vermiculite-MMN medium.

Table 2. Effect of inoculation on the growth of jack pine seedlings and mycorrhizal development in containers after 20 wk.

	Height^{1} (cm)	Shoot^{1} weight (mg)	Root^{1} weight (mg)	Shoot root ratio	Percent^{2} infection $\bar{X}$	SD	Total short roots seedling^{-1}	Weight of^{1} roots with 300 short roots (mg)	Short roots mg^{-1} root weight
Thelephora terrestris	5.4^{a}	291^{bc}	295^{abc}	.99	100	0	1395	65^{bc}	5.1
Laccaria proxima	4.5^{a}	220^{abc}	259^{ab}	.85	99^{c}	2	1695	48^{abc}	6.7
Hebeloma sp.	5.1^{a}	247^{abc}	435^{3}	$.59^{3}$	99^{c}	2	1047	$-^{3}$	2.5^{3}
E-strain	4.4^{a}	179^{a}	191^{a}	.95	91^{bc}	7	1442	42^{abc}	8.0
Cenococcum geophilum	4.8^{a}	237^{abc}	313^{bc}	.79	57^{abc}	14	2367	39^{abc}	8.1
Pisolithus tinctorius	4.4^{a}	191^{ab}	296^{abc}	.66	54^{abc}	11	1234	72^{c}	4.5
Astraeus hygrometricus	4.6^{a}	abc 236abc	200^{a}	1.21	48^{ab}	17	1090	51^{abc}	6.6
Lactarius paradoxus	5.1^{a}	266abc	288^{abc}	.93	32^{a}	11	2596	34^{a}	9.3
Sphaerosporella brunnea	4.5^{a}	251^{abc}	344^{bc}	.75	17^{a}	27	2525	41^{abc}	8.0
Amphinema byssoides	5.2^{a}	322^{c}	396^{c}	.80	0		3336	37^{ab}	9.0
Hydnum imbricatum	4.3^{a}	257^{ab}	348^{bc}	.74	0		2766	38^{ab}	8.4
Tricholoma flavovirens	4.2^{a}	226^{abc}	331^{bc}	.69	0		2483	41^{bc}	8.0
Control	4.9^{a}	259^{abc}	372^{c}	.69	0		3083	37^{ab}	8.5

(cont'd)

Table 2 (continued).

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[1] Data analyzed by one-way ANOVA and differences among means tested for using Scheffé pairwise comparisons.

[2] Data analyzed by Kruskal-Wallis test. Values in each column followed by the same letter do not differ significantly ($p = .05$).

[3] Roots colonized by *Hebeloma* impossible to clean of debris due to the heavy development of extramatrical mycelium.

growth of Amphinema could be seen around the roots. The large weight of the roots infected by Hebeloma was due to the production of copious amounts of mycelium and the inability to clean off the planting mixture. The root systems of seedlings infected with E-strain and Astraeus were significantly smaller than the uninfected control seedlings.

The mean number of short roots produced varied considerably among treatments. Those treatments lacking infection or with very low infection levels generally had the largest number of short roots. With the exception of Cenococcum, plants with greater than 48% infection had one-half to one-third the number of short roots found in the control treatment. The data on number of short roots/mg suggests that mycorrhizal infection reduced the initiation of short roots. However, the relationship, if it exists, is much less clear when the root weight of laterals plus the 300 short roots on them is considered. Similar values were found for heavily and noninfected root systems.

Nine of the 12 fungi tested successfully formed mycorrhizae with jack pine (Table 2). All of those tested, with exception of the Amphinema isolate, successfully formed mycorrhizae in monoxenic culture (Danielson, unpubl. data). The most aggressive fungi were Thelephora, Laccaria, Hebeloma and E-strain. Sphaerosporella infected a minor portion of the short roots and apparently survived marginally in the planting mixture as 5 of the 10 seedlings were devoid of any infection. Isolates of Amphinema, Hydnum and Tricholoma failed to initiate mycorrhizal infection.

Mycorrhizae formed by Pisolithus were monopodial to coralloid, with stout elements vinaceous buff, floccose with abundant extramatrical mycelium (EMM) and hyphal strands. Laccaria proxima + jack pine mycorrhizae were branched dichotomously 1-3 times with stout elements, cream to fulvous, with a small amount of EMM. Thelephora mycorrhizae were composed of stout elements branched dichotomously 1 to 3 times, pallid to pale snuff brown or fawn to bay or purplish chestnut, the mantle surface was smooth and compact with sparce EMM and occasional mycelial strands 80 to 100 μm in diameter.

The mantle in plain view was a tight textura intricata, the hyphae were 2 to 3 μm diameter, ochraceous and clamped. Jack pine + S. brunnea mycorrhizae were very difficult to detect as little morphological changes of the short roots occurred. The mycorrhizae were simple or with dichotomously branched long elements, most bay but occasionally pallid or black, colour due to roots rather than S. brunnea, infected short roots very slightly inflated. At 500X it could be seen that the mantle was absent but that the Hartig net was well developed. Hebeloma + jack pine mycorrhizae were composed of long elements branching up to 3 times, mycelial strands lacking, EMM very abundant forming a white mass of mycelium around the roots which effectively bound the planting mixture. Mycorrhizae formed by Astraeus, Cenococcum and E-strain were typical for the respective combinations (Danielson, 1982; unpubl. data).

2.5 DISCUSSION

Although it has been clearly shown that certain fungi may benefit survival and growth of tree seedlings (Marx, 1980) problems concerning the inoculation are equally clear. At present, only a few of the thousands of symbionts of conifers have been tested for their effects on forest regeneration. Proper evaluations cannot be made until procedures are developed which allow the development of a wide range of symbiont-host mycorrhizal associations under greenhouse conditions and subsequent field testing of the stability and efficacy of such relationships. At present, the failure of such relationships to develop within the greenhouse, e.g. with Hydnums and agarics such as Suillus and Tricholoma (this study; Molina, 1980), preclude definitive evaluations.

In this inoculation study, fungi known as "weed" species or those common in nursery operations performed the "best", i.e. resulted in high levels of mycorrhizal formation. Thelephora, Laccaria, and E-strain fungi are all too well known as symbionts of nursery or container-grown seedlings (Laiho, 1965; Molina, 1977; Marx et al., 1981). Far less is known of the effects on the host of fungi fruiting in natural stands of conifers, e.g. Suillus, Tricholoma, Russula, Cortinarius, Lactarius and the hypogeous symbionts. This deficiency cannot be corrected and properly tested until inoculation procedures are developed which result in mycorrhizal infection within the greenhouse and field evaluations are conducted.

The cause of the failure of Amphinema, Hydnum and Tricholoma to form mycorrhizae is unknown. After 16 weeks it was apparent that infections by these fungi were not occurring and 5 ml of a slurry inoculum made from 15 to 20 colonies was injected into each of the 85 replicate containers. However this method of inoculation also failed although it has been shown to work with other fungi (see Part 5). It was felt that Hydnum imbricatum would be successful as it produces abundant small chlamydospores in culture (Danielson, unpubl. data) which should aid in survival. However this did not prove to be true or else conditions were unfavorable for Hydnum to initiate infection. Fertilizer levels were low and it seems unlikely that nutrient levels inhibited infection. However, the pH of the planting mixture was about 6.4 and may have caused a reduction in the survival of the symbionts.

The presence of mycorrhizae had little effect on plant growth when the experiment was terminated. This situation is ideal for the subsequent outplanting and the evaluation of mycorrhizal effects on host performance. However, had the fertilizer level not been increased after four months, seedlings infected with E-strain, Laccaria and Thelephora would have been smaller than seedlings in the other treatments. Shaw et al. (1982) also found that infection by Laccaria laccata (Scop. ex Fr.) Berk. & Br. and Hebeloma crustuliniforme (Bull. ex St. Amans) Quél. resulted in a growth depression. They suggested that the reduced growth of the host was due to preferential utilization of carbohydrates by the fungi. Although the biomass and C utilization of symbionts has never been quantified, the collection of fruit bodies of Thelephora in this study showed that they amounted to 59 mg dry weight per seedling. This was about 10% of the weight of the host, thus representing a substantial C drain. Using higher levels of fertilization apparently increased the amount of photosynthate produced and eliminated the growth depression.

Infection also had the potential to reduce the number of short roots produced, possibly altering the capacity for nutrient and water uptake.

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3. THE EFFECTS OF ECTOMYCORRHIZAL FUNGI ON THE SURVIVAL AND GROWTH OF JACK PINE SEEDLINGS OUTPLANTED ON AMENDED OIL SANDS TAILINGS - FIRST SEASON RESULTS

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3.1 ABSTRACT

Jack pine seedlings inoculated with 9 fungi or left uninoculated were outplanted on extracted oil sands amended with muskeg peat and clay. Nonmycorrhizal seedlings produced under operational conditions were planted for comparison. The experimental seedlings were smaller than the operational seedlings both prior to outplanting and at the end of the first growing season. Inoculation had none or very small effects on survival and shoot and root growth during the first growing season. Thelephora terrestris, Laccaria proxima, Hebeloma sp. and E-strain readily colonized new roots that extended into the spoil. Astraeus hygrometricus, Pisolithus tinctorius, Cenococcum geophilum, Lactarius proxima and Sphaerosporella brunnea infected only a minor portion of the new short roots. Ten species of indigenous fungi infected 4% of the short roots extending into the spoil. E-strain fungi were the most common and aggressive of the indigenous fungi.

3.2 INTRODUCTION

Establishment of ectomycorrhizal woody plants on extracted oil sands will be dependent on many chemical and physical factors as well as the presence of compatible and beneficial symbionts. These fungi may be indigenous in peat or surficial overburdens, spontaneously infect container-grown seedlings during nursery production or be intentionally introduced via inoculation procedures. Fungi indigenous in amended oil sands may be so sparcely distributed that mycorrhizal-free seedlings planted in them may become infected only very slowly or the fungal species present may be relatively ineffective. Fungi which invade seedlings in the greenhouse prior to outplanting may be aggressive colonizers but some at least, are less effective than species native to stressed sites (Marx, 1980). Thus it appears that careful symbiont selection and deliberate inoculation of ectomycorrhizal hosts could benefit the establishment of woody plants on oil sands tailings.

To date, very few fungi have been evaluated under field conditions for enhancement of host growth and most research has concentrated on *Pisolithus tinctorius* (Pers.) Coker & Couch. In that this species is not known to form mycorrhizae in the prairie provinces or northern Canada it appears to be wholly justified to test species that occur on native plants near oil sands mining operations.

The objectives of this outplanting study were (1) to compare the survival and growth of jack pine seedlings infected with a wide range of native ectomycorrhizal fungi, as well as *Pisolithus*, planted on oil sand tailings amended with peat and mineral overburden, (2) to compare the success of experimentally produced seedlings with those produced in an operational greenhouse, (3) to determine the persistence of the introduced fungal species and to determine the degree of short root infection by indigenous symbionts.

3.3 MATERIALS AND METHODS

Details of inoculation procedures and seedling production are given in Part 2. Although inoculation failed with three of the

12 fungi, all treatments were used. In addition, seedlings were obtained from the Syncrude nursery. These were raised under a higher nutrient regime and were cold hardened after 12 wk but were 20 wk old, as were the experimental seedlings when planted. The experimental seedlings were moved directly from the greenhouse to the field site and all seedlings were planted on June 16 and 17. The Syncrude seedlings were significantly ($p = .05$) taller (9.1 cm) and heavier (shoots = 440 mg, roots = 251 mg) than the experimental seedlings (see Part 2). The Syncrude seedlings were nonmycorrhizal (based on evaluations of a total of 3000 short roots on 10 seedlings).

The planting site was the Syncrude tailings pond dyke near Fort McMurray, Alberta (57°5'N, 111°45'W). The climate is cool continental with long cold winters and short cool summers. The growing season lasts 60 to 80 days with a maximum day length of 18 h.

The extracted oil sands, from which the containment dyke was built, was amended with 15 cm of stockpiled muskeg peat and 10 cm of clay mineral overburden. The amendments were mixed coarsely into the sand with a cultivator. At the time of planting, the area was completely devoid of vegetation. The peat was from a muskeg site and was stockpiled in 1976 and planted with a mixture of grasses in 1977 to prevent erosion. The peat was spread in December 1981 and cultivated in May 1982. The area was not fertilized.

The experimental plot consisted of 27 rows planted down the face of the dyke. Seedlings were planted 0.5 m apart with 1 m spacing between rows. Seventy five seedlings of each treatment, except *Tricholoma*, were planted in two randomly selected rows i.e. each row contained 36 to 38 seedlings. One row of *Tricholoma flavovirens* inoculated seedlings were planted as half the seedlings were retained in the greenhouse for other purposes. The seedlings were planted June 16 and 17, 1982, each one marked with a stake and numbered consecutively. On Sept. 14, five even-numbered seedlings from each row were randomly selected and dug up to determine growth and mycorrhizal characteristics after one growing season. Seedlings which did not survive were recorded. The plot design and sampling pattern is given in Appendix Table 1.

Analysis of growth and mycorrhizal characteristics were similar to that performed prior to outplanting. Shoot heights were measured, branches counted and the shoots dried at 80°C and weighed. Roots extending from the planting plug were clipped off and used for mycorrhizal determinations. Roots in the plug were dried and weighed separately from those extending into the spoil. The extending roots were cut into 2 to 3 cm segments and segments were randomly selected until 300 short roots per seedling were rated for infection. Infections other than those caused by the introduced species were characterized by taxonomic affinity, morphology, amount of extramatrical mycelium, presence of rhizomorphs or cystidia and pigmentation. Mycorrhizae caused by unknown symbionts were plated on MMN^+ or benomyl-MMN to further characterize them.

3.4 RESULTS

Seedlings grown under operational greenhouse conditions were larger and heavier than experimental seedlings after 3 mo in the field (Table 1). There was only one significant difference of height and weight parameters among the inoculation treatments. Only three seedlings died during the first growing season although many were in poor condition and it is expected that they will not survive the winter. Root weight outside the plug of seedlings inoculated with Hebeloma was significantly larger than those in the Astraeus, Pisolithus and control treatments. The roots were clean of debris and the differences were not due to errors of including non-root material. Root growth outside the planting plug was apparently least with uninoculated seedlings and seedlings inoculated with Pisolithus or Astraeus. The total number of short roots (i.e. potential infection units) was also least with the latter three treatments and greatest in the Syncrude and Cenococcum inoculated seedlings.

Excluding the Syncrude treatment, shoot weight of all experimental seedlings increased 3-fold during the first growing season. Total root weight increased 2-fold during the same period (excluding Syncrude and Hebeloma). Root mass within the planting

Table 1. Growth characteristics of inoculated jack pine seedlings after one growing season on amended oil sands[1].

	Height[2] (cm)	Branches[3] seedling^{-1}	Shoot[2] weight (mg)	Root weight (mg)		No. short roots outside	Short roots mg root^{-1}	Wt. of root[4] with 300 short roots (mg)
				Total[2]	Outside plug			
Thelephora terrestris	6.8^{a}	2.1^{ab}	1005^{a}	797^{ab}	213^{ab}	1102	5.3	60^{ab}
Laccaria proxima	5.6^{a}	4.3^{ab}	827^{a}	611^{a}	103^{ab}	730	6.1	42^{ab}
Hebeloma sp.	6.4^{a}	1.6^{a}	809^{a}	762^{ab}	226^{b}	978	4.3	68^{b}
E-strain	6.0^{a}	2.5^{ab}	592^{a}	563^{a}	155^{ab}	1017	6.1	47^{ab}
Cenococcum geophilum	6.2^{a}	2.3^{ab}	836^{a}	714^{ab}	165^{ab}	1644	9.0	33^{a}
Pisolithus tinctorius	5.7^{a}	2.6^{ab}	486^{a}	409^{a}	59^{a}	480	7.7	35^{a}
Astraeus hygrometricus	6.1^{a}	1.8^{ab}	801^{a}	516^{a}	88^{a}	703	8.3	34^{a}
Lactarius paradoxus	5.5^{a}	1.5^{a}	729^{a}	628^{a}	108^{ab}	719	8.3	43^{ab}
Sphaerosporella brunnea	5.6^{a}	2.8^{ab}	658^{a}	665^{a}	116^{ab}	823	6.7	43^{ab}

(cont'd)

Table 1 (continued).

Amphinema byssoides	5.6^{a}	3.3^{ab}	858^{a}	719^{ab}	118^{ab}	759	6.5	42^{ab}
Hydnum imbricatum	5.8^{a}	2.5^{ab}	866^{a}	721^{ab}	130^{ab}	823	7.4	40^{ab}
Tricholoma flavovirens[5]	6.3	2.8	848	504	167	1438	8.6	39
Syncrude	14.1^{b}	6.0^{b}	2580^{b}	1400^{b}	315^{b}	2297	7.3	42^{ab}
Control	6.1^{a}	1.8^{ab}	646^{a}	636^{a}	95^{a}	703	6.3	40^{ab}

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[1] Data analyzed by one-way ANOVA and difference detected by Scheffé pairwise comparisons. Values in each column followed by the same letter not significantly different ($p = .05$).

[2] Data ln Y transformed.

[3] Data ln (Y + 1) transformed.

[4] Analysis of covariance with the number of roots counted as the covariate.

[5] Not included in analysis as only five replicates done.

plug increased substantially while in the field, but only 20% of the total root weight was that of extended roots. The height of the experimental seedlings increased from 4.8 to 5.9 cm while in the field, while the Syncrude seedlings increased from 9.1 to 14.1 cm in height. Branches were formed while in the field as the experimental seedlings increased from 1.1 to 2.3 branches per seedling.

Roots growing into the spoil were successively colonized by Thelephora terrestris, Laccaria proxima, Hebeloma sp. and E-strain (Table 2). Of the other five fungi which infected the roots in the greenhouse, all but Sphaerosporella brunnea colonized between 1 and 6% of the short roots on extended laterals.

Indigenous mycorrhizal fungi were present in the amended oil sand but did not result in large scale infections (Table 2, Appendix Table 2). Overall, 3.9 ± 11.5% (x ± SD) of the short roots from all treatments were infected with indigenous symbionts. Indigenous fungi occurred on 42 (31%) of the total seedlings with a total of 55 indigenous occurrences. A maximum of three fungi were found on a single seedling. The most common indigenous species was the E-strain (Table 3). It was found on 16% of the outplanted seedlings and infections resulted in a substantial portion of the root systems being colonized. The I-type, the hyaline Basidiomycete and the Rhizopogon-like species occurred on about half as many seedlings and were much less successful in colonizing the root systems than the E-strain. Six other species were detected on only one seedling each and except for the unknown Ascomycete, they infected only a few roots. Half the indigenous species were Basidiomycetes and half were Ascomycetes. Only the Rhizopogon-like species produced mycelial strands and all had hyaline hyphae except for E-strain and Cenococcum.

In order to determine if position within the plot influenced seedling growth or mycorrhizal development, data were analyzed by dividing the plot into four horizontal strips and three vertical strips. Neither position up the slope nor along the length of the dyke influenced shoot growth (Table 4). When the infection data was analyzed in a similar manner, it appeared a position in the

Table 2. Infection of jack pine seedling roots growing out from planting plugs into amended oil sands by introduced and indigenous fungi.

	Percent infection by:			
	All fungi		Introduced species	
	$\bar{x}$	SD	$\bar{x}$	SD
Thelephora terrestris	75	18	75	18
Laccaria proxima	43	23	42	23
Hebeloma sp.	48	13	48	12
E-strain	93	11	93	11
Cenococcum geophilum	3	4	2	3
Pisolithus tinctorius	14	16	6	4
Astraeus hygrometricus	24	22	4	6
Lactarius paradoxus	9	19	1	2
Sphaerosporella brunnea	.4	1	0	0
Amphinema byssoides	6	12	NA	NA
Hydnum imbricatum	.5	1	NA	NA
Tricholoma flavovirens	3	4	NA	NA
Syncrude	5	14	NA	NA
Control	0	0	NA	NA

NA = not applicable.

Table 3. Infection of jack pine seedlings by mycorrhizal species indigenous to the amended oil sands.

Symbiont	Number of seedlings infected (totals 135)	Percent of total seedlings colonized	Percent infection of colonized seedlings
E-strain	22	16.2	31.5
I-type ascomycete	10	7.4	3.4
Hyaline basidiomycete	9	6.6	7.9
Rhizopogon-like	8	5.9	1.8
Cenococcum geophilum	1	.7	4.3
Unknown basidiomycete	1	.7	2.3
Unknown ascomycete	1	.7	45.3
Floccose basidiomycete	1	.7	2.9
Unknown affinity 1	1	.7	2.1
Unknown affinity 2	1	.7	.3

Table 4. Effect of position within the dyke plot on shoot growth of jack pine. Syncrude seedlings excluded from the analysis. Data analyzed by dividing the plot into four horizontal strips and three vertical strips.

	Horizontal strips				
Vertical strips	Top	Upper-middle	Lower-middle	Bottom	Means
	shoot weight (mg)				
Left	833	752	825	776	797[a]
Middle	827	1000	821	685	834[a]
Right	835	729	698	612	718[a]
Means	832[a]	827[a]	781[a]	692[a]	

Data analyzed by one-way ANOVA.

middle of the plot favored mycorrhizal development (Table 5). However rows in the left one-third of the plot were planted with seedlings that lacked mycorrhizae in the greenhouse, while those in the centre had high infections in the greenhouse. To further test position effect, the two rows of each species were compared with t tests with regard to infection. This test indicated there were no significant ($p = .05$) effects of position on the dyke slope on mycorrhizal infection.

3.5 DISCUSSION

Although it is generally assumed that mycorrhizae benefit survival and growth of plants on mine spoils (Marx, 1975), mycorrhizae had no effects during the first growing season in this study. As infection levels of roots growing in the spoil were substantial with four of the test species of fungi, it appears that either the fungi were ineffective or the time was too short for the trees to respond. After one year, Navritil et al. (1981) were able to show that inoculation with Pisolithus resulted in increased growth of jack pine. However, Riffle and Tinus (1982) found little benefit of inoculation of two pine species after one year in the field in North Dakota. This may have been due to the low level of infection by the introduced species, which was not the case in the present study.

The experimental seedlings used were well below the size desired for outplanting (Carlson, 1979). Tests conducted since the initiation of the outplanting study show that higher levels of fertilizer can be used without impairing mycorrhizal development (see Part 8). Future outplanting studies will utilize larger seedlings which may result in better first season growth and perhaps allow the effects of the mycorrhizal condition to be expressed.

As expected, the amount of indigenous inoculum compatible with jack pine was very low. If seedlings lack mycorrhizae when planted on peat amended tailings, success may be impaired as there is insufficient inoculum present to infect a significant portion of the roots. This indicates that inoculation is desirable and further,

Table 5. Effect of position within the dyke plot on mycorrhizal infection of jack pine seedlings.

Vertical strips	Horizontal strips Top	 Upper-middle	 Lower-middle	 Bottom	Means
	shoot weight (mg)				
Left	1.1	.2	6.1	15.1	5.6[a]
Middle	46.9	35.8	61.5	51.8	49.0[b]
Right	21.8	15.7	11.6	15.8	16.2[a]
Means	23.3[a]	17.2[a]	26.4[a]	27.6[a]	

Data 2 arcsin $\sqrt{p}$ transformed and analyzed by one-way ANOVA. Differences among means detected by Scheffé pairwise comparisons. Values of the row and column means followed by the same letter do not differ significantly (p = .05) .

that inoculations are likely to be successful (i.e. the introduced fungi are able to spread to new root(s) due to the absence of competing symbionts. Even fungi which infected only a small portion of the extending roots during the first season, such as Pisolithus and Cenococcum, may expand onto a significant portion of the root system in the second growing season as competition appears to be minimal.

In general, the indigenous fungi may not be well adapted to conditions on the dyke, as most "species" infected only a very small percentage of the short roots of plants they occurred on. It can be assumed that most or all of indigenous symbionts came from a muskeg site where moisture and temperature conditions, as well as hosts, differed greatly from those on the dyke. Tests should be done to determine the relative benefits of species indigenous to the peat and introduced species from drier sites.

The indigenous species were similar or identical to those found in other studies of peat (see Part 6). The E-strain indigenous to the peat differed from the introduced E-strain in that mantle cells were large (up to 10 to 14 m) and intracellular infection did not occur. However cultures were typical for E-strain fungi (Danielson, 1982). In that E-strain inoculum was fairly common and that infections spread through the root systems readily, E-strain fungi are to be contended with in mycorrhizal aspects of oil sands reclamation. Efforts should be made to determine the range of host response to a variety of E-strain fungi. This should also include several hosts as E-strain fungi can infect a very wide spectrum of woody plants (Laiho, 1965).

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4. A COMPARISON OF MYCORRHIZAL DEVELOPMENT OF CONTAINER-GROWN JACK PINE SEEDLINGS EITHER OUTPLANTED ON AMENDED OIL SANDS TAILINGS OR REARED IN THE GREENHOUSE

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4.1 ABSTRACT

Container-grown jack pine seedlings were either planted on an oil sands mine containment dyke amended with muskeg peat and clay or were planted in the same material in the greenhouse. The seedlings were nonmycorrhizal at the time of planting. At the end of one growing season, plant growth and mycorrhizal development were determined. Shoot growth and weight of roots extending from the planting plug were substantially greater in the greenhouse than in the field. Seedlings in the field remained nonmycorrhizal whereas two-thirds of short roots of the greenhouse seedlings were infected by five symbionts. Greenhouse baiting techniques appear to have limited quantitative predictive value but can be used to more rapidly determine the spectrum of fungal symbionts present in amended mine spoil than is possible under field conditions.

4.2 INTRODUCTION

In order to determine if mycorrhizal inoculum is present in a particular soil or minespoil, it is necessary to grow the appropriate species of plant in the material. This can be done either in the field or in the greenhouse. Growing the plants in the greenhouse has the advantage of convenience and ensuring that if different treatments are used, they are all subjected to the same conditions. However, conditions in the field and the greenhouse may differ so strongly that rates of mycorrhizal development and the symbionts involved may be substantially different.

Schoenberger and Perry (1982) have used what they termed a greenhouse bioassay to determine the mycorrhizal potential of forest soils where clear-cutting and burning had taken place. They suggested that results of the bioassay may not be extrapolated to the field but that the technique was valid for comparing the effects of disturbance. The objective of this study was to determine the degree of similarity between mycorrhizal formation of seedlings grown under field and greenhouse conditions.

4.3. MATERIALS AND METHODS

Container-grown jack pine (Pinus banksiana Lamb.) were grown as described in Part 2. The control treatment trees planted on the Syncrude dyke were used for comparison with seedlings retained in the greenhouse. Extracted oil sand amended with muskeg peat and clay was collected from five sites immediately adjacent to the outplanting plot. The samples consisted primarily of peat which was packed into 12.5 cm pots and planted with uninfected seedlings identical to those planted on the dyke. The pots were placed in the greenhouse which had supplemental lighting to provide a 20 h daylength with a minimum of 3.5 klx. Temperature ranged from 18 to 25°C. Ten replicate seedlings were watered three times weekly with deionized water and ten replicates were watered once weekly and fertilized twice weekly with a solution of 100 mg l^{-1} of 15:15:18 fertilizer in deionized water. Plants were removed from the field after 13 wk and from the greenhouse after 9 wk and weights and mycorrhizal status determined (Part 2).

4.4 RESULTS AND DISCUSSION

Shoot weights of seedlings grown in the greenhouse were significantly larger than those grown in the field (Table 1). Fertilization did not significantly affect either shoot or root growth. Root weights within the planting plug apparently reached a maximum as weights in all three treatments were the same. However, the amount of extending roots was much greater under greenhouse conditions than in the field. In terms of plant nutrition and survival, the amount of extending roots is very likely much more important than roots in the plug. Extending roots will serve to anchor the seedlings and prevent frost-heaving, exploit a large volume of peat for nutrients and water and greatly increase the chance of roots encountering mycorrhizal inoculum. Shoot/root ratios may be misleading when the root system exists in two distinct compartments; rather emphasis should be placed on the amount and mycorrhizal condition of extending roots.

Differences between mycorrhizal development of greenhouse and field-grown seedlings were clear and dramatic (Table 2). Nearly two-thirds of the short roots of seedlings grown in the greenhouse were mycorrhizal, whereas no mycorrhizae were detected on seedlings from the field. Fertilization had no effect on mycorrhizal formation. Five species of fungi were involved as symbionts but one, referred to here as the hyaline basidiomycete, was clearly dominant. It is fairly certain that all of these species originated in the peat-spoil as other jack pine seedlings grown in the greenhouse simultaneously remained nonmycorrhizal. In addition, the hyaline Basidiomycete, the I-type and the Rhizopogon-like fungus were all detected in other seedlings in the outplanting trial (Part 3).

The success of the hyaline Basidiomycete appears to be due to two factors; inoculum density and the ability to rapidly spread through the root system once an initial infection occurs. Three of the 20 greenhouse seedlings were not infected by the hyaline basidiomycete indicating that few propagules were present in each pot. But where infection was present it exceeded 30% except on one seedling. In contrast, the Rhizopogon-like symbiont infected 8

Table 1. Growth characteristics of containerized jack pine seedlings either reared in the greenhouse or in the field.

	Field	Greenhouse Fertilized	Greenhouse Unfertilized
Shoot weight (mg)	646^{a}	1823^{b}	1363^{b}
Root weight in plug (mg)	540^{a}	640^{a}	608^{a}
Root weight, total (mg)	636^{a}	1067^{b}	1002^{ab}
Shoot/root ratio	1.0	1.8	1.3

Data analyzed by one-way ANOVA and differences tested by Scheffé pairwise comparisons. Values in each row followed by the same letter do not differ significantly ($p = .05$).

Table 2. Mycorrhizal development of containerized jack pine seedlings either reared in the greenhouse or in the field.

	Percent infection and standard deviation					
	Field		Greenhouse Fertilized		Greenhouse Unfertilized	
Symbiont	$\overline{X}$	SD	$\overline{X}$	SD	$\overline{X}$	SD
Total infection	0	0	67	28	60	37
I-type Ascomycete	0	0	1	2	2	5
Hyaline Basidiomycete	0	0	62	26	52	39
Rhizopogon-like	0	0	.3	.4	5	7
White floccose	0	0	3	7	.7	2
Hyaline cystidial	0	0	0	0	.4	.7

seedlings but only an average of 5.8% of the short roots were infected. Both the white floccose and the I-type were found in 6 seedlings each, infecting 6.7 and 7.7% of the short roots respectively. Thus, it appears these latter three fungi were less able to spread through the root system than was the hyaline Basidiomycete. The pattern of producing small localized infections appears to be typical of Rhizopogon-like fungi found in peat (Part 6).

In the field where the hyaline basidiomycete occurred, it did not spread extensively (Part 3). As this species is indigenous to muskeg sites, it may be that the frequent watering in the greenhouse favored rapid spread. Conditions on the dyke were droughty throughout the summer and moisture probably limited plant growth and may have affected mycorrhizal development.

The differences between mycorrhizal infections in the greenhouse and the dyke may have been due to carbohydrate levels in the roots (Marx et al., 1977; Dixon et al., 1981) or the amount of extending roots. Poor growth in the field may have resulted in low root carbohydrate levels which may have reduced infectivity. In a previous study in which jack pine grew very poorly in unfertilized peat, infection was less in the unfertilized plants than when fertilizer was applied (Danielson et al., 1982). In that seedlings in the greenhouse had 4-fold more weight of extending roots than those in the field, the latter root systems were much less likely to encounter the sparcely distributed inoculum.

The use of a greenhouse baiting or bioassay technique with substrates containing low levels of inoculum may be useful for comparing different treatments but appear to have limited quantitative predictive value. However, mycorrhizae developing in the greenhouse were formed by the same species observed in a larger outplanting study on the same substrate. Seedlings grown for a one season equivalent in the greenhouse may reflect the mycorrhizal condition of seedlings grown for two or more seasons in the field. If so, considerable time can be saved in determining mycorrhizal potential of materials containing low levels of inoculum in short season climates by using a baiting technique.

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5. THE EFFECTIVENESS OF MYCELIAL SLURRIES FOR THE INOCULATION OF CONTAINERIZED JACK PINE SEEDLINGS

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5.1. ABSTRACT

Mycelial slurries prepared from agar plates of 15 species of ectomycorrhizal fungi were used to inoculate seven week old container-grown jack pine. Six species formed mycorrhizae at levels similar to those obtained using the standard vermiculite-peat solid carrier. These included *Thelephora terrestris*, *Laccaria proxima*, *Hebeloma* sp., *Pisolithus tinctorius*, *Cenococcum geophilum* and an E-strain fungus. The use of a slurry enhanced mycorrhizal formation by *Sphaerosporella brunnea* but the technique failed with *Lactarius paradoxus* and *Astraeus hygrometricus* which were successfully inoculated using peat-vermiculite. Species of *Tricholoma*, *Suillus*, *Amphinema* and *Hydnum* failed to initiate infections regardless of inoculation technique. The use of a mycelial slurry has the advantage of saving considerable time in inoculation preparation and should be useful for experimental purposes.

5.2 INTRODUCTION

A variety of techniques have been used to inoculate seedlings with mycorrhizal fungi including the use of infested soil, spores and, most commonly, mycelium grown in a mixture of peat and vermiculite (Marx, 1980; Marx and Kenney, 1982; Trappe, 1977). Although Trappe (1977) states that the use of pure cultures in peat and vermiculite guarantees success, failures do occur, even with fungi that grow rapidly in culture (Danielson, unpub. data; Molina, 1980; Shaw and Molina, 1980). The failure of many fungi to infect roots prevents the evaluation of potentially useful associations in field situations as well as encouraging the use of a few, easily manipulated fungi.

Difficulties in obtaining infection of jack pine with several species of fungi introduced into containers (see Part 2) led to attempts in using other inoculation methods. The simplest method of inoculation with pure cultures is the maceration of mycelium and its introduction into the root zone. This technique has been used successfully with Hebeloma (Debaud et al., 1981), Laccaria (Brown and Sinclair, 1981) and Rhizopogon (Theodorou, 1980). The technique has the advantage over using solid inoculum carriers in that seedlings can be inoculated when short roots are present rather than prior to germination. Thus it was felt that it would be useful to determine if a simple application of a mycelial slurry to seedlings when short roots were present would result in mycorrhizal infection.

5.3 MATERIALS AND METHODS

Jack pine (Pinus banksiana Lamb.) seedlings were grown in 65 cm^3 Cone-tainers (Ray Leach, Canby, Oregon) using a 1:1 (v/v) mix of peat and vermiculite. Fertilizer was added twice per week to saturation with 80 mg l^{-1} of 10:42:10 during the second week and 100 mg/l of 15:15:18 for the remainder of the growth period. Sequestrene Fe (56 mg l^{-1}) and micronutrients were added twice per week. Conditions for growth were similar to those for the outplanting study (Part 2). In addition to the 12 species used in the outplanting study, an additional species of both Suillus and Tricholoma as well as Amphinema byssoides, were included. The symbionts of jack pine were grown in MMN agar plates and from 2-15 colonies depending on colony size, were blended in a Virtis tissue homogenizer for 15-25 sec. The mycelial suspensions were centrifuged and washed once in deionized water and resuspended in water. Each of five replicate 7 wk old seedlings for each fungus was inoculated by injecting 10 ml of the suspension into the center of the container. When the seedlings were 18 wk old the roots were washed and examined for infection. If any infection was present, the root system was cut into 2-3 cm segments and segments randomly selected until 300 short roots had been rated for infection. Roots and shoots were dried and weighed to determine plant response to infection. The pH of the peat-vermiculite when seedlings were harvested was 5.9-6.0.

5.4 RESULTS AND DISCUSSION

Due to the low level of fertilizer used, shoots were small in all inoculation treatments (Table 1). Shoot-root ratios were less than one in every combination but with one. This was similar to the results found with the seedlings used in the Syncrude outplanting trial in which a higher fertilization level was used (see Part 2). There were no significant effects of inoculation on shoot growth except between Hebeloma and Suillus tomentosus. Root weight of Laccaria was significantly smaller than four other combinations. These effects are likely due to the heavy infection of jack pine by Laccaria and Hebeloma which act as a carbohydrate sink and under limiting nutrient conditions, reduce the growth of the host. A depression in growth of container-grown Sitka spruce by Hebeloma crustuliniforme (Bull. ex St. Amans) Quél. and Laccaria laccata (Scop. ex Fr.) Berk. & Br. has been observed by Shaw et al. (1982).

Infection by Laccaria and Hebeloma also reduced the total number of short roots per plant in comparison to the control (Table 2). The frequency of short root initiation was highest in the control and significantly lower in seedlings inoculated with Hebeloma. Inoculation with Thelephora, Pisolithus and Cenococcum also resulted in significantly less short roots per unit weight of the total root

Table 1. Shoot and root growth of jack pine inoculated with mycelial slurries of 15 symbionts at age 7 wk and grown in containers for an additional 11 wk under a low fertilizer regime.

Species	Shoot weight (mg) $\bar{x}$	Shoot weight (mg) SD	Root weight (mg) $\bar{x}$	Root weight (mg) SD
Control	160^{ab}	37	180^{ab}	31
Thelephora terrestris Ehrhart ex Fr.	155^{ab}	37	190^{ab}	30
Laccaria proxima Boudier	154^{ab}	6	131^{a}	19
Hebeloma sp.	119^{a}	33	206^{ab}	53
E-strain	121^{ab}	19	179^{ab}	18
Pisolithus tinctorius (Pers.) Coker & Couch	178^{ab}	37	191^{ab}	33
Cenococcum geophilum Fr.	183^{ab}	29	223^{b}	32
Sphaerosporella brunnea (Alb. & Schw. ex Fr.) Svrcek & Kubicka	161^{ab}	32	210^{b}	45
Astraeus hygrometricus (Pers.) Morg.	158^{ab}	10	205^{ab}	22
Lactarius paradoxus Beardslee & Burlingnam	135^{ab}	39	171^{ab}	32
Suillus tomentosus (Kauff.) Sing., Snell & Dick	186^{b}	28	206^{ab}	51
Suillus umbonatus Dick & Snell	179^{ab}	43	227^{b}	25
Tricholoma flavovirens (Pers. ex Fr.) Lundell	146^{ab}	29	192^{ab}	26
Tricholoma pessundatum (Fr.) Quel.	128^{ab}	29	179^{ab}	28
Hydnum imbricatum L. ex Fr.	164^{ab}	14	214^{b}	27
Amphinema byssoides (Fr.) John Erikss.	155^{ab}	32	196^{ab}	48

Data was analysed by 1-way ANOVA, Tukey pairwise comparisons. Values in each column followed by the same letter do not differ significantly at $p = .05$.

Table 2. Short root characteristics and mycorrhizal infection of container grown jack pine seedlings inoculated with a mycelial slurry at age 7 wk and grown an additional 11 wk.

	Percent infection		Short roots per plant		Short roots per mg root weight	
Species	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Control	0^{a}	0	2232^{b}	515	12.4^{c}	2.2
Thelephora terrestris	99^{e}	.3	1370^{ab}	307	7.3^{ab}	1.8
Laccaria proxima	100^{e}	0	1250^{a}	319	9.6^{abc}	2.3
Hebeloma sp.	79^{cd}	17	1137^{a}	443	5.4^{a}	1.1
E-strain	98^{de}	1	1692^{ab}	279	9.4^{abc}	.8
Pisolithus tinctorius	39^{b}	10	1380^{ab}	346	7.2^{ab}	1.1
Cenococcum geophilum	42^{b}	26	1746^{ab}	288	7.8^{ab}	.9
Sphaerosporella brunnea	56^{bc}	8	2001^{ab}	263	9.8^{bc}	2.3

Data was analyzed by one-way ANOVA and differences among means determined by Scheffé multiple contrasts for pairwise comparisons. Percent infection data transformed by 2 arcsin $\sqrt{p}$. Values in each column followed by the same letter do not differ significantly at $p = 0.05$.

Seven of the 15 fungi listed in Table 1 successfully formed mycorrhizae with jack pine (Table 2). Levels of infection were comparable to those obtained using peat-vermiculite inoculum (see Part 2)) although infection by Cenococcum and Pisolithus was somewhat lower using a slurry and infection with Sphaerosporella was higher using a slurry. The variation in infection by Sphaerosporella was much less with the slurry than with peat-vermiculite with means and standard deviations being 17 ± 27% for the peat-vermiculiite and 56 ± 8% for the slurry. Thus the inoculum of Sphaerosporella appeared to be barely able to survive in the peat-vermiculite until susceptible roots were available for infection but were readily able to internally colonize roots when placed directly on them. Of the 12 species common to both studies, only Lactarius and Astraeus formed mycorrhizae when peat-vermiculite was used and failed when a slurry was used. Astraeus is very sensitive to fragmentation of the mycelium (Visser and Danielson, unpub. data) and it is likely that this reduced the number of viable propagules to critical levels. The reason for the failure of Lactarius is unknown as it survives fragmentation well (Visser and Danielson, unpubl. data). Amphinema byssoides, Tricholoma pessundatum and Hydnum imbricatum failed using both techniques. All formed mycorrhizae with jack pine under pure culture synthesis conditions (Danielson, unpub. data). No infection was observed for any of the other species tested except two seedlings inoculated with Suillus tomentosus had less than 15 mycorrhizae typical of this species (i.e. < 1%). The failure of Suillus species to form mycorrhizae with container-grown seedlings has been observed previously (Molina, 1980). It appears that the mycelium of some species cannot survive in the planting mix for even short periods. Though data is lacking, death is probably caused by antagonism by elements the soil microflora. When spores of Suillus tomentosus were used to inoculate jack pine seedling under conditions similar to those used here, mycorrhizae were formed (Danielson and Visser, unpubl. data). This suggests that the physical and chemical environment within the tubes was favorable for mycorrhizal formation by Suillus if infective propagules were present. A decrease in the inoculum potential of Cenococcum with increasing time spent in containers has been shown by Graham and Linderman (1981). It seems likely that the mycelium of some species are especially sensitive to antagonist organisms when not in a mycorrhizal association.

Although Marx and Kenney (1982) dismissed using mycelial suspensions to inoculate seedlings and Riffle and Maronek (1982) did not mention it, it appears that the use of suspensions have at least one advantage over peat-vermiculite for some experimental situations. Considerable time can be saved by using suspensions as inoculum plates can be started after seedlings are started rather than 3 mo or more before. In addition, for fungi which have survived poorly in planting mixes, such as Sphaerosporella, inoculation with a suspension when short roots are present may be more effective than when peat-vermiculite is used.

The failure of certain fungi to initiate infection when either applied in a solid carrier at the time of planting or when mycelium is applied directly to susceptible short roots remains a serious problem to be solved. Unless a broader spectrum of fungi can be field tested for effects on host performance, the question of what are the "best" symbionts cannot be resolved.

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6. EFFECT OF STOCKPILING MUSKEG PEAT ON THE ECTOMYCORRHIZAL DEVELOPMENT OF JACK PINE.

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6.1 ABSTRACT

A greenhouse baiting technique was used to determine the effects of stockpiling muskeg peat on the infectivity of peat with regards to ectomycorrhizal formation with jack pine. Seedling growth was poor apparently due to nutrient deficiencies and the high pH of the stockpiled peat. Fertilization of the stockpiled peat was ineffective in stimulating plant growth unless the seedlings were mycorrhizal. Stockpiling resulted in reduced infection levels, fewer mycorrhizal seedlings and fewer symbionts as compared to undisturbed muskeg. The most important fungal symbionts in the stockpile were E-strain fungi, a cystidial Ascomycete and a hyaline Basidiomycete. Ascomycetes accounted for 37% of the infected short roots and 32% of the mycorrhizae had cystidia on the mantle. Rhizomorphs were rarely formed. The most aggressive fungi were those mentioned above and the least aggressive were a *Rhizopogon*-type and *Cenococcum geophilum*.

6.2 INTRODUCTION

During the course of surface mining of bitumen-bearing oil sands in northern Alberta, the muskeg peat deposits are routinely stockpiled for reclaiming the extracted tailings. Stockpiling mineral topsoil during mining operations in the U.S. and the resultant effects on VA mycorrhizal inoculum has received some attention (Rives *et al*., 1980; Gould and Liberta, 1981) but data on either the stockpiling of soils containing ectomycorrhizal inoculum or peat soils is totally lacking. The stockpiling of topsoil generally reduces the VA infectivity with time but not necessarily to levels which will detrimentally affect plant growth or survival after application of the stored soil to spoils as compared to freshly spread soil.

However, peat deposits differ substantially from mineral soils in their inherent mycorrhizal components and stockpiling peat may have more serious consequences on mycorrhizal associations than stockpiling mineral soil. The native vegetation of muskeg peat bogs is largely coniferous and ericaceous and therefore VA mycorrhizal inoculum is probably rare. The drastic changes in soil moisture between undisturbed bogs and the reclamation sites where the peat is applied suggests that the indigenous fungi may be ill-adapted to the new conditions. Stockpiling may reduce species diversity and thus the ecological and physiological versatility of mycorrhizae. In addition, nutrient availability may be altered by stockpiling and create nutrient conditions which may favour nonindigenous symbionts.

Whereas VA fungi are not host specific, ectomycorrhizal fungi are often host specific although some species may form mycorrhizae with a wide taxonomic range of higher plants. These fungi, e.g. *Cenococcum* and E-strain, are found over broad geographic ranges, encompassing many types of ecosystems. It is thus unlikely that a soil supporting any ectomycorrhizal hosts would completely lack inoculum for nonindigenous hosts unless the hosts exhibited strong ectomycorrhizal specificity, e.g. *Alnus* spp. However, the effectiveness of these broad-spectrum hosts is unknown. It has been suggested that host-specific ectomycorrhizal fungi may be more effective, i.e. benefit the host more, than broad spectrum fungi (Mikola, 1970). This suggestion has yet to be critically tested but it is worthy of consideration rather than simply recording overall infection rates and relating these to the success of reclamation treatments incorporating inoculum-bearing amendments. A first step in testing potential efficiencies is to determine the spectrum of compatible symbionts present in peat amendments and to attempt to characterize these taxonomically and with regard to adaptive morphological features.

It is thus of interest to determine the fate of ectomycorrhizal inoculum in stockpiled peat and to evaluate the characteristics of the mycorrhizae as they may relate to the efficiency of nutrient and water uptake in reclaimed tailings subjected to critical stresses. The objectives of this study were to determine:

(1) the potential of undisturbed and stockpiled peat for initiating mycorrhizal infections of a prime reclamation candidate, jack pine;

(2) the morphological characteristics of the ectomycorrhizae;

(3) the identity of the symbionts indigenous to the peat;

(4) the inherent growth potential of the peat for jack pine seedlings.

6.3 MATERIALS AND METHODS

The muskeg peat was located on the Syncrude Oil Sands Lease (57°5'N, 111°45'W) in northern Alberta. The peat was about 2 m deep and supported several ectomycorrhizal plants including eastern larch (Larix laricina (DuRoi) K. Koch) and swamp birch (Betula pumila L. var. glandulifera Regel). Black spruce (Picea mariana (Mill.) BSP) occurred at one end of the bog and small willows (Salix sp.) were widely scattered in the area. Ericaceous plants, especially Labrador tea (Ledum groenlandicum Oeder), were abundant as were feather mosses and hummocks of sphagnum mosses. VA mycorrhizal hosts were very rare except in two samples which were taken in old cutlines which were vegetated with grasses.

The peat was stripped from a portion of the bog and stored in the period between Dec. 1980 and Feb. 1981 to form a stockpile about 300 m wide and 3 m deep. When sampled in the second week of September, 1981, the only ectomycorrhizal hosts were widely scattered seedlings of aspen (*Populus tremuloides* Michx.) and willow. The major colonizer was lamb's quarters (*Chenopodium album* L.) which is nonmycorrhizal (Hirrel *et al.*, 1978). Also present was fireweed (*Epilobium angustifolium* L.).

At the two sampling locations both surface and subsurface samples were taken. On the stockpile, the surface was subject to drying and the 0-15 cm depth was sampled to represent this layer. Due to difficulty in inserting the Macauly peat sampler at some sample points on the stockpile the subsurface samples varied from 50-100 cm deep but all samples were moist and well below the level subject to drying. In the undisturbed bog the surface samples were taken by hand to a depth of 15 cm below the bases of the feather or sphagnum mosses. The material sampled was moist, but due to the dry summer conditions, never saturated. The tops of all mosses on the hummocks were dry. The surface layer sampled was largely a mass of roots (many of which were ericaceous) and active ectomycorrhizae were frequently abundant. The subsurface samples taken with the Macauly sampler were usually from the 50-100 cm depth and were much moister and contained far fewer roots than the surface samples. About 200 cm^3 of peat was taken for each sample.

One 250 m long transect across the stockpile and one 250 m transect across the undisturbed bog were established. Every 10 m along each transect line a surface sample was taken at a randomly selected point 1 to 5 m to the left of the line. The subsurface samples were taken at random points 1 to 5 m to the right of the transect to eliminate depth dependency. Thus, a total of 25 replicates were taken for each of the four depth-disturbance combinations.

The samples were stored at 5°C for about 5 wk and then each sample was thoroughly mixed and one 65 cc Leach Cone-tainer filled and planted with a single pregerminated jack pine seedling. The seedlings were placed in a greenhouse with day length supplemented with 3.5 klux lights to give a 15 h daylength for the first 8 wk and 18 h daylength for the next 9 wk. The seedlings were watered twice weekly for the first 10 wk after which they were fertilized twice weekly with a solution containing 125 mg/l of 15:15:18 Plant Prod complete fertilizer. After a total of 17 wk, the seedlings were placed at 5°C, shoots harvested and dried, and the roots evaluated for mycorrhizal development and then dried at 80°C. All short roots were rated for mycorrhizal infections, taxonomic affinity of the symbiont and morphological characters of the mantle and extramatrical mycelium. Isolations on benomyl-MMN agar were used to further characterize the symbionts (Danielson _et al_., 1983).

In that seedlings in all treatments grew poorly, the entire experiment was repeated with samples that had been stored at 5°C for 6 months. Daylength was extended to 20 h and 100 mg/l of Plant Prod 15:15:18 was added throughout the 15 wk growth period. After 10 wk, growth in the stockpiled peat was still poor so additional Fe (56 mg l^{-1} Sesquestrene) and micronutrients (1 ml of micronutrient stock, Arnon, 1938) was added. Only mycorrhizal development of seedlings grown in stockpiled peat were evaluated in the second experiment. Five randomly chosen seedlings were used to determine seedling size on the undisturbed samples. The pH of the samples were determined with 10 g peat in 20 ml water. Initially the values for the materials were: undisturbed surface pH 6.5, undisturbed subsurface pH 5.7, stockpile surface pH 7.4 and stockpile subsurface pH 7.7.

6.4 RESULTS

Growth of the jack pine seedlings was poor in all of the peat sources (Table 1) and the application of fertilizer after 10 wk growth appeared to have no effect. Both root and shoot growth were significantly greater in peat from the undisturbed bog than in stockpiled peat. However, the total number of short, or feeder,

Table 1. Characteristics of jack pine seedlings grown in peat from a muskeg bog and peat stockpiled for eight months (Experiment I).

	Depth (cm)		
	0-15	50-100	
Peat Source	Shoot weight (mg)		Row means
Undisturbed	36	32	34^{b}
Stockpile	21	20	21^{a}
Column means	28^{a}	26^{a}	
	Root weight (mg)		
Undisturbed	26	34	30^{b}
Stockpile	20	22	21^{a}
Column means	23^{a}	28^{b}	
	Short roots/seedling		
Undisturbed	353	276	265^{a}
Stockpile	300	320	310^{b}
Column means	277^{a}	298^{a}	

Shoot and root weight analyzed by two-way ANOVA, short roots/seedling analyzed by analysis of covariance with adjustment for total root weight. Differences between means tested by Scheffé pairwise comparison. Values (geometric means) within each set followed by the same letter in each row or column do not differ significantly (p = .05).

roots was greater in the stockpiled peat than in the bog peat. The depth from which the peat was obtained had little effect on the weight of the seedlings. The poorest appearing seedlings were in the subsurface stockpile peat and unlike seedlings in the other treatments, failed to form any needle fascicles. No height growth beyond the cotyledon stage occurred in any seedling.

The degree of mycorrhizal development was much greater in seedlings grown in the undisturbed peat than in stockpiled peat (Table 2). Depth of peat down to 0.5-1 m had no significant effect on infection rates although the data suggest that inoculum was less plentiful on the stockpile surface than in the body of the stockpile. The number of seedlings infected, total numbers of infections and the number of symbiont species were all much reduced by stockpiling the peat. The number of infections was estimated by assuming that all the internal colonization by each species on each seedling was due to a single infection event.

A total of 31 taxa of symbionts of jack pine were recorded from all the peat sources (Table 3, Appendix Table 1). Few of these taxa could be assigned to known genera or species and most were given descriptive trivial names which correspond to names in Parts 2 and 3. Two criteria were used to denote the importance of the various taxa, the percent of all the infected short roots infected by each taxon and the percent of the seedlings infected by each taxon. In order to determine post-infection behavior of the symbionts, i.e. aggressiveness, the degree (%) of infection of each seedling internally colonized by each taxon was determined.

Of the total roots infected, 37% were infected by Ascomycetes and Ascomycetes composed 25% of the symbiont species. Accordingly, Basidiomycetes were responsible for 61% of the short root infections. Eight species (25%) had pigmented hyphae and these eight formed 13% of the mycorrhizae. With regard to external features, 8% of the mycorrhizae were glabrous, 32% had cystidia and 26% formed extensive extramatrical mycelium. Rhizomorphs and crystalline deposits on the hyphae were found with only two species and these formed 2.4% of the mycorrhizae.

Table 2. Mycorrhizal infection of jack pine seedlings grown in undisturbed muskeg and stockpiled peat (Experiment I).

Peat Source	Depth (cm)	% Infection[1] $\overline{X}$	SD	Total Seedlings	Number of seedlings infected	Total number of infections	Number of species surviving
Undisturbed	0-15	78^b	23	24	24	89	25
Undisturbed	50-100	53^b	36	25	24	58	15
Stockpile	0-15	1^a	4	23	3	3	3
Stockpile	50-100	15^a	29	20	7	8	6

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[1] Data analyzed by Kruskal-Wallis test.

Table 3. Types of mycorrhizae developing in the greenhouse on jack pine seedlings grown in muskeg peat from an undisturbed bog and from a stockpile.

Taxa (no.)	External features[1]	Hyphal pigments[2]	% of total infected short roots[3]	% of seedlings infected		% infection of colonized seedlings	
				Bog	Stock	$\overline{X}$	SD
Ascomycetes							
I-type (1)	Cystidia	-	22.4	40.8	4.7	34	31
E-strain (3)	EMM	+	5.8	6.1	2.3	63	43
Cenococcum (5)	EMM	+	.5	16.3	0	3	3
T. angularis (9)	[EMM]	-	1.2	2.0	0	63	0
Black asco (11)	EMM	+	.3	2.0	0	14	0
Coarse asco (13)	Glabrous	-	6.3	10.2	2.3	34	27
No. 22	[EMM]	+	.05	2.0	0	2	0
No. 25	EMM	+	.02	2.0	0	.7	0
Basidiomycetes							
Hyaline basid (12)	[EMM]	-	28.8	40.8	9.3	40	36
Golden floccose (6)	EMM	-	8.5	34.7	0	14	14
Tibiiform (10)	Cystidia	-	4.4	2.0	0	97	0
Tomentella I (2)	[EMM]	+	4.3	34.7	0	10	10
Tomentella II (21)	Cystidia	+	2.3	10.2	0	15	15

(cont'd)

Table 3. (cont'd)

Yellow cystidia (4)	Cystidia	-	3.1	24.5	0	11	17
Rhizopogon-like (7)	EMM + C + R	-	1.8	30.6	2.3	5	7
Rhizopogon-like (8)	EMM + C + R	-	.6	4.1	0	12	14
White floccose (30)	EMM	-	3.3	0	2.3	45	0
White floccose (14)	EMM	-	.4	2.0	0	24	0
No. 16	[EMM]	-	1.0	2.0	0	58	0
No. 18	EMM	-	.04	2.0	0	2	0
Tom-like (20)	Glabrous	+	.03	2.0	0	2	0
No. 24	EMM	-	.3	2.0	0	10	0
No. 26	EMM	-	3.3	8.2	0	20	25
No. 27	EMM	-	.02	2.0	0	1	0
No. 29	Glabrous	-	.9	2.0	0	30	0
Dense floccose (31)	EMM	-	.5	0	2.3	14	0
Unknown affinity							
No. 15	?	-	.1	4.1	0	3	3
No. 17	Glabrous	-	1.1	2.0	0	30	0
No. 19	EMM	-	.8	4.1	0	16	18
No. 23	[EMM]	-	.2	2.0	0	6	0
No. 28	?	?	.05	2.0	0	2	0

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[1] Presence of cystidia on the mantle, EMM = extramatrical mycelium, C = crystals on the hyphae, R = rhizomorphs or mycelial strands, [ ] = sparse EMM.

[2] Individual hyphae with definite brown pigments.

[3] A total of 10,091 infected short roots.

The species forming the most mycorrhizae were the I-type Ascomycete (I-subtype of Dominik, 1962) and the hyaline Basidiomycete. These two species accounted for 50% of the infections. Also of importance were E-strain fungi and the coarse Ascomycete. It is likely that the coarse Ascomycete is related to the E-strain but it did not grow well enough in culture to confirm the relationship. These same four taxa were also responsible for a majority of the infection initiated in the stockpiled peat. In the undisturbed peat, the average number of species per seedling were 3.8 in the surface and 2.2 in the subsurface.

Post-infective aggressiveness could only be reliably estimated for species which occurred four or five times or more. Those species best able to internally colonize jack pine roots were the E-strain, coarse Ascomycete, I-type and the hyaline Basidiomycete. Particularly inefficient in spreading was Rhizopogon (7) which occurred 16 times but only infected an average of 5% of the short roots. Cenococcum geophilum also occurred frequently but was very slow to extend from the original infection point. The golden floccose (6) and Tomentella species were intermediate in aggressiveness.

Fertilization throughout the growing period resulted in increased seedling growth but the seedlings were still small in comparison to seedlings grown in commercial peat (compare with 1x treatment in Part 7). Shoot weights (± standard deviations) of seedlings grown in the undisturbed peat were 76 ± 15 mg - control surface, 38 ± 8 mg - control subsurface, 166 ± 64 mg - fertilized surface and 114 ± 26 mg - fertilized subsurface. In stockpile peat, fertilization increased shoot weight 63% and root weight 76% and decreased short roots per seedlings 11% (Table 4). Seedlings that did not receive any fertilizer all appeared to be N deficient and/or P deficient. Those that were fertilized appeared to be deficient in N or were a healthy green colour, i.e. fertilization alone did not correct the nutrient deficiencies (see below). The application of additional micronutrients and Fe did not result in any apparent changes in the plants. It is very likely that the pH of the

Table 4. Characteristics of jack pine seedlings grown in stockpiled peat unfertilized or with fertilizer added twice weekly (Experiment II).

	Depth (cm)		
	0-15	50-100	
Fertilized	Shoot weight (mg)		Row means
-	25	27	26^a
+	37	42	40^b
Column means	31^a	34^a	
	Root weight (mg)		
-	23	26	24^a
+	33	45	38^b
Column means	27^a	34^b	
	Short roots/seedling		
-	368	377	372^b
+	349	308	329^a
Column means	358^a	343^a	

Shoot and root weight analyzed by two-way ANOVA, short roots/seedling analyzed by analysis of covariance with adjustment for total root weight. Differences between means tested by Scheffé pairwise comparison. Values (geometric means) within each set followed by the same letter in each column or row do not differ significantly ($p = .05$).

stockpiled peat was responsible for the poor growth. The pH (± standard deviation) of the fertilized peats at the end of the growth period were 7.4 ± .4 - undisturbed surface, 6.3 ± .4 - undisturbed subsurface, 7.8 ± .3 - stockpile surface and 7.9 ± .2 - stockpile subsurface.

Mycorrhizal infection was clearly enhanced by fertilization of both depths of peat (Table 5). Once fertilizer was applied it became apparent that the surface peat was less infective than the subsurface peat. This was true for percent short roots infected, number of seedlings infected and total number of infections. The number of species detected did not vary with depth.

The dominant symbiont was the hyaline Basidiomycete and the only other fungi which infected a large number of short roots were the I-type and E-strain fungi (Table 6). Fertilization did not result in a change in the species that infected jack pine but increased the number of seedlings infected. As in the previous experiment, E-strain, hyaline Basidiomycete and I-type were the most aggressive symbionts.

Of the 24 surviving fertilized seedlings in the surface peat it was noted that 14 exhibited N deficiency symptoms and 10 seedlings were healthy green. Eight of the 10 healthy seedlings were mycorrhizal whereas only one deficient seedling was infected (15%). In the subsurface fertilized peat all 14 healthy green seedlings were mycorrhizal. A comparison of sizes of infected and noninfected (including those with less than 30% infection) seedlings was made using a t-test. In the surface, infected seedlings were significantly ($p \leq 0.05$) larger (54 mg) than noninfected seedlings (32 mg). The subsurface peat was almost identical, 34 mg versus 54 mg, with the infected seedlings being significantly larger.

6.5 DISCUSSION

The stockpiled peat was a poor growth medium for jack pine seedlings whether fertilized or not. The difference in pH between the undisturbed muskeg and the stockpile may well account for the differences in growth between the two sources. At nearly pH 8, the

Table 5. Mycorrhizal infection of jack pine seedlings grown in fertilized and non-fertilized stockpiled peat (Experiment II).[1]

Depth (cm)	Fertilized	% Infection		Total Seedlings	Number of seedlings infected	Total number of infections	Number of species surviving
		$\bar{X}$	SD				
0-15	-	3^a	8	23	3	3	3
0-15	+	22^b	33	24	10	11	6
50-100	-	5^a	15	22	4	5	3
50-100	+	51^c	42	21	17	21	6

[1] Data analyzed by Kruskal-Wallis test.

Table 6. Types of ectomycorrhizae developing on jack pine seedlings planted in stockpiled peat which was either fertilized or not.

Taxa (No.)	Presence of external features[1]	Hyphal pigments[2]	% of total infected short roots[3]	% of seedlings infected		% infection of colonized seedlings	
				-Fert	+Fert	$\bar{X}$	SD
Ascomycetes							
I-type (1)	Cystidia	-	14.1	2.2	8.9	63	30
E-strain (3)	EMM	+	15.1	0	6.7	57	39
Coarse asco (13)	Glabrous	-	1.5	2.2	0	44	0
Basidiomycetes							
Hyaline basid (12)	[EMM]	-	57.2	6.7	35.6	54	35
Tomentella I (2)	[EMM]	+	2.0	2.2	4.4	19	19
Rhizopogon-like I (7)	EMM + C + R	-	1.0	4.4	2.2	10	10
Dense floccose (23)	EMM	-	3.8	0	6.7	20	16
Unknown affinity							
No. 24	EMM	-	5.1	0	2.2	35	23
No. 32	?	-	.01	0	2.2	.2	0

[1] Presence of cystidia on the mantle, EMM = extramatrical mycelium readily apparent, R = rhizomorphs, C = crystals, [ ] = sparse EMM.

[2] Individual hyphae with definite brown pigments.

[3] A total of 7267 short roots infected.

availability of P and micronutrients would be reduced which may account for the small growth response to fertilizer. It may be of considerable importance that the mycorrhizal plants appeared to respond to fertilization while the nonmycorrhizal plants did not respond. However it is possible that the larger plants become infected because they were more susceptible rather than mycorrhizae enhancing growth. It seems more likely however that mycorrhizae enhanced nutrient uptake, probably N, which resulted in better growth. McVean (1963) also found pine seedlings to grow very poorly in peat unless the plants were mycorrhizal. Mycorrhizal seedlings took up more P and N than nonmycorrhizal seedlings and McVean (1963) concluded that the enhanced growth was a result of becoming infected rather than a cause of infection. He credited mycorrhizae as having a major influence on the field success of scots pine planted on open moorlands and bogs.

There was no clear indication that any one symbiont was superior to another. However, it was observed that the only seedling grown in the stockpile peat in the first experiment with healthy colour was heavily infected with E-strain. In the fertilized stockpile peat, the largest seedling produced (shoot weight 162 mg) was 100% mycorrhizal with the E-strain. This suggests that E-strain fungi may be particularly beneficial but further testing is required.

The spectrum of fungal symbionts was typical for peat soils. In other baiting experiments and greenhouse studies the I-type Ascomycete, E-strain, hyaline Basidiomycete, Rhizopogon-like and Tomentella are constant associates of jack pine seedlings (Danielson et al., 1982a, b; Danielson et al., 1983, see Part 3). These fungi also developed mycorrhizae in the field on extracted oil sands amended with peat which had been stockpiled for five years (Part 2). Although additional observations are necessary to determine the role of these fungi in field reclamation sites, there is little doubt that field performance evaluations are warranted.

It is unknown if the mycorrhizal status of jack pine grown in the greenhouse reflects the status of ectomycorrhizal plants in the native bog. It is tempting to extrapolate the data obtained in this study to muskeg bogs but it is impossible without field observations. It would be of considerable interest to determine the properties of the mycorrhizae in undisturbed muskeg to determine the similarity of taxonomy and morphology between indigenous plants in the greenhouse and native plants in the field.

Although the amount of mycorrhizal inoculum was less on the surface of the stockpile than in the interior, this probably has little practical significance. The portion of a pile that is subject to moisture and temperature extremes is a relatively small volume and would be mixed and diluted with the unexposed peat when applied to tailings sand. Stockpiling peat results in a less infective and less species diverse growth medium as compared to the surface meter of native bog. These reductions may be due to dilution with deeper layer or death of propagules following stockpiling. Regardless, it would be expected that sufficient inoculum is present to eventually infect plants in the field. However, the time required to form infections of sufficient magnitude to influence host performance might be considerable.

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7. EFFECT OF STOCKPILING MUSKEG PEAT ON THE VESICULAR-ARBUSCULAR MYCORRHIZAL DEVELOPMENT IN SLENDER WHEATGRASS

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7.1 ABSTRACT

Vesicular-arbuscular (VA) mycorrhizal development was determined for slender wheatgrass (Agropyron trachycaulum (Link) Malte.) grown in peat from two depths (0-15 cm, 50-100 cm) in an undisturbed muskeg bog and peat stockpile. Mycorrhizal development was negligible in all but the 0-15 cm deep peat from the undisturbed bog. Plants grown in the 0-15 cm undisturbed peat became infected if the peat had originally suported a VA host, but if a non-VA host had been present (e.g. larch, ericaceous plants), slender wheatgrass exhibited very little infection. Stockpiling for 6 years increased VA inoculum levels, but at a very slow rate. When inoculum of the VA fungus, Glomus aggregatum, was introduced into the inoculum-deficient stockpiled peat, slender wheatgrass demonstrated a positive growth response. Fertilization tended to override this growth response. The lack of VAM inoculum in the peat being used in the revegetation programs, may have adverse effects on the establishment of plant species highly dependent on the mycorrhizal relationship.

7.2 INTRODUCTION

The excavation of oil bearing sand from the Athabasca tar sand deposits in northern Alberta currently involves removal of the muskeg peat and overburden overlying the sand. The peat deposits have an average thickness of 1 m (depths range from 0 to over 4.5 m) and are drained prior to being stripped and stockpiled (Thomas, 1981). The stored muskeg is subsequently used for the reclamation of mined land, in particular, tailings sand which is used to dyke the tailings pond.

The extraction process for removing the oil from the sand is so efficient that the resultant tailings sand is virtually pure sand devoid of all organic matter and nutrients. Therefore, reclamation guidelines set up by the Alberta government require the incorporation of 15 cm muskeg and 10 cm clay into the tailing sand prior to revegetation. The addition of muskeg to the sand improves the plant growth medium by supplying nutrients and by increasing water and nutrient retentivity.

In addition to ameliorating the chemical/physical properties of the extracted sand, muskeg peat, because it is removed from the rooting zone and because it is highly organic in nature, should also be effective in improving the biological properties by introducing both heterotrophic microorganisms active in decomposition and nutrient cycling processes and serving as a source of symbiotic microorganisms such as N_2 fixing bacteria and mycorrhizal fungi. It has become widely recognized that the symbiotic N_2 fixing bacteria such as *Rhizobium* and the actinorrhizal organisms improve the nitrogen nutrition of the legumes and certain woody shrub species such as alder, whereas the mycorrhizal fungi through their extensive mycelium increase the ability of the plants to absorb P. There is also some evidence that the mycorrhizal symbiosis may be instrumental in the water relations of the plant (Safir *et al*., 1971; Allen *et al*., 1981), thereby improving its ability to survive drought. Since the mycorrhizal condition may be crucial in alleviating both nutrient and water stresses (conditions often found in recently revegetated tailings dykes) it may be necessary to ensure successful mycorrhizal infection to guarantee survival and maintenance of those plant species used in a reclamation program which are highly dependent on the mycorrhizae.

It is highly likely that the plant species currently used for revegetation purposes will require the presence of both vesicular-arbuscular mycorrhizal (VAM) fungi and ectomycorrhizal fungi. Grasses and legumes which are commonly planted to stabilize slopes and control erosion are predominantly VA mycorrhizal while many of the tree species such as jack pine are ectomycorrhizal. Many of the woody shrubs which have been proposed as candidates for future revegetation are either VA mycorrhizal (e.g. *Amelanchier alnifolia* Nutt., *Prunus pensylvanica* L.f., *Rosa acicularis* Lindl.) or ecto- or VA mycorrhizal (e.g. *Alnus crispa* (Ait.) Pursh., *Shepherdia canadensis* (L.) Nutt., *Eleagnus commutata* Bernh.). Therefore, if the establishment of an efficient mycorrhizal system on the roots of these plants is considered a necessary component of a revegetation strategy information is required on the following:

(1) the incorporation and distribution of both VAM and ectomycorrhizal fungi in undisturbed peat bogs prior to stockpiling.

(2) the effect of draining and stockpiling this peat on the qualitative and quantitative aspects of the mycorrhizal component. Drainage of the peat prior to stockpiling may eliminate or reduce inoculum levels, while the process of stockpiling may either bury inoculum originally concentrated in the rooting zone or dilute high inoculum surface soil with the deeper soil lacking inoculum. Storage of peat over an extended period of time, particularly in the absence of host plants upon which the mycorrhizal fungi depend, may not only reduce inoculum levels, but may also eradicate those organisms with a low survival potential. Studies by Rives *et al*. (1980) and Gould and Liberta (1981) have demonstrated that stockpiling topsoil for future reclamation of coal mine sites results in a significant decrease in VAM infection potential.

To obtain information regarding the effect of stockpiling on the composition, quantity and relation of the mycorrhizal fungi, a greenhouse study was conducted whereby an ectomycorrhizal species (jack pine) and a VA mycorrhizal species (slender wheatgrass) were grown in peat sampled from two depths in an undisturbed bog and an adjacent peat stockpile. Plant growth parameters and the mycorrhizal status of the roots were subsequently determined and where possible the mycorrhizal fungi involved in the symbiosis were identified. The results obtained for the VA mycorrhizal component of the study are presented here.

7.3 MATERIALS AND METHODS

7.3.1 Effect of Stockpiling Peat for 8 Months on VA Mycorrhizal Development in Slender Wheatgrass

Peat samples were randomly removed from two depths (0-15 cm; 50-100 cm) in an undisturbed peat bog and a peat stockpile on the Syncrude site near Fort McMurray, Alberta. The vegetation on the bog was predominantly eastern larch, swamp birch, Labrador tea and feather and sphagnum mosses while the peat stockpile which had been built eight months previously was largely unvegetated.

A more complete description of the sampling sites and sampling methods is reported in Danielson *et al*. (Part 5, this report). Each sample was thoroughly mixed, packed into a 65 cm^3 Leach Cone-tainer and planted with one pre-germinated slender wheatgrass seedling. The seedlings were placed in a greenhouse and grown under the light conditions outlined by Danielson *et al*. (Part 5, this report). No fertilizer was added during the course of the experiment. Plants were grown for 12 weeks and then assessed for the following parameters:

(1) shoot and root production - shoots were clipped at the shoot-root interface, dried at 80°C and weighed. Roots were placed on a 1 mm sieve, washed free of soil and approximately 0.15 g was subsampled for determination of mycorrhizal status. The remaining roots were dried at 80°C and the moisture content calculated for each root sample was used to extrapolate to total dry root produced.

(2) mycorrhizal status - subsampled roots were cleared and stained with .01% trypan blue (Phillips and Hayman, 1970). Five root samples from each of the four treatments (i.e. undisturbed bog 0-15

cm; undisturbed bog 50-100 cm; stockpiled peat 0-15 cm and stockpiled peat 50-100 cm) were randomly chosen, mounted on slides and evaluated for mycorrhizal infection by the method described by Zak and Parkinson (1982).

To determine the species of VA mycorrhizal fungi, those peat samples which produced the greatest level of infection in the grass roots after 12 weeks growth were sieved for spores by the sucrose centrifugation method (Allen et al., 1979; Smith and Skipper, 1979) outlined by Zak et al. (1982) for peat amended minespoil.

Selected physical, chemical and biological characteristics were also assessed for five randomly chosen peat samples from each of the four treatments. Loss on ignition, pH, microbial activity and biomass C were determined as described by Visser et al. (1982a, b) while moisture retention at .03 MPa was measured by the pressure plate extraction technique.

7.3.2 Effect of Stockpiling Peat for 6 Years on Mycorrhizal Infection in Slender Wheatgrass

A preliminary assessment of the slender wheatgrass roots grown in undisturbed bog peat and peat stockpiled for 8 months revealed that VA mycorrhizal inoculum levels were negligible unless a VA host had been growing in the peat when the sample was taken. Since peat from a six year old stockpile which had been vegetated with a grass mixture was used to amend the experimental plot in the jack pine outplanting study (see Part 2, this report), it was decided to sample this peat to determine if any changes in VAM inoculum levels had occurred over the storage time.

Five peat samples (0-20 cm deep) were randomly removed from the northern boundary of the experimental plot. These were transported to the lab where they were subsampled and the subsamples mixed thoroughly. Twenty, 65 cm^3 Leach Cone-tainers were packed with peat and one pre-germinated slender wheatgrass seedling was planted in each Container. The seedlings were divided into two treatments - ten seedlings received Plant Prod fertilizer at a rate of 100 ppm 15-15-18 twice per week, and 56 ppm sequestrene twice per week while the remaining seedlings received deionized water only. Plants were grown in the greenhouse under a 20 h daylength, with a minimum of 3.5 klux light intensity.

The parameters measured after 9 weeks growth included shoot and root production and mycorrhizal status of the roots. The methods used in these assessments were identical to those described in section 7.3.1.

7.3.3 Growth of Slender Wheatgrass in 8 Month Old Stockpiled Peat Mixed with Vesicular-Arbuscuar Mycorrhizal Fungal Inoculum

Since peat from the 8 month old stockpile contained very low levels of VA inoculum, an experiment was conducted to determine if the addition of VA inoculum would improve the growth of slender wheatgrass under fertilized or unfertilized conditions.

A pot culture of *Glomus aggregatum* Schenk & Smith (a common VA fungus having a wide distribution in Alberta) was established by growing slender wheatgrass in sand which had been inoculated with this fungus. The grass was grown in the greenhouse (20 h daylength; 3.5 klux light intensity) and was fertilized twice weekly with 100 mg l^{-1} of 15-15-18 Plant Prod. After 4.5 months shoots were clipped while the roots were checked for mycorrhizal infection and the root/sand inoculum stored at 5°C.

Peat from the 50-100 cm depth in the 8 month old stockpile was bulked and separated into two batches. One batch was mixed 50/50 (v/v) with root/sand inoculum from the *G. aggregatum* pot culture while the other batch was mixed 50/50 (v/v) with autoclaved root/sand inoculum to serve as a control. Twenty Leach Cone-tainers were packed from each batch and one pre-germinated slender wheatgrass seedling was planted in each container. Seedlings were grown in the greenhouse under the conditions described in section 6.3.1. Five seedlings from each treatment (inoculated, fertilized; uninoculated, fertilized; inoculated, unfertilized; uninoculated, unfertilized) were sampled when the plants were 4 weeks and 10 weeks old. Shoot and root weights were determined by the methods described in 6.3.1. The presence of mycorrhizal infection in each treatment was assessed by clearing and staining root subsamples (see section 6.3.1 for techniques) and scanning the samples under the dissecting microscope.

7.4. RESULTS

7.4.1 Effect of Stockpiling Peat for 8 Months on VA Mycorrhizal Development in Slender Wheatgrass

Data on the pH, moisture retentivity, loss on ignition and microbial activity and biomass in the undisturbed bog and peat stockpile are summarized in Tables 1 and 2. Stockpiling caused an increase in soil pH, particularly at the 50-100 cm depth, and a decrease in organic matter and moisture holding capacity (Table 1). Microbial respiration in the stockpiled peat was lower than that in the surface soil in the undisturbed bog, but not less than the respiration measured in peat from the 50-100 cm depth of the bog (Table 2). The upper 0-15 cm peat in the undisturbed bog also harbored the largest amount of microbial biomass C, with no differences in biomass occurring in peat from the lower depth of the bog and peat from the stockpile (Table 2).

Slender wheatgrass produced the heaviest shoots when grown in peat from the 0-15 cm depth of the bog or stockpile (Table 3). Root productivity was highest in plants grown in the 0-15 cm bog peat (Table 3). The shoot/root ratios of plants in all treatments were low (Table 3) inferring that the peat lacked sufficient nutrients for healthy plant growth.

The total length of root produced by the slender wheatgrass in the undisturbed peat and stockpiled peat was not significantly different (Table 4). Mycorrhizal infection of the roots was highest in the undisturbed 0-15 cm peat and negligible in the stockpiled peat and 50-100 cm bog peat (Table 4). Although the mean percent mycorrhizal infection in the undisturbed surface peat was 14.5%, it is worthwhile noting that of the 5 root samples analyzed, only two samples contained VA mycorrhizal fungi. These two root samples were from peat which, when sampled from the bog, had a predominantly grass vegetation cover, the roots of which may have served as a source of VA inoculum. Slender wheatgrass grown in peat which originally had

Table 1. Selected chemical and physical characteristics of undisturbed muskeg peat and stockpiled peat^{1}. (peat stockpiled for 8 months).

		Sampling depth (cm)		Row
Soil parameter	Peat source	0-15	50-100	means
pH	Undisturbed	6.5^{a}	5.7^{a}	
	Stockpile	7.4^{b}	7.7^{c}	
Moisture retention (% H_2O wet wt basis) at .03 MPa	Undisturbed	83.3	77.8	80.5^{b}
	Stockpile	75.2	67.7	71.5^{a}
	Column means	79.3^{b}	72.8^{a}	
Organic matter (% loss on ignition)	Undisturbed	85.6^{b}	89.1^{b}	
	Stockpile	55.9^{a}	42.1^{a}	

[1] Data analyzed by two-way ANOVA (MSE = 109991.8 for pH; 26.3 for moisture; 114.0 for organic matter) and Scheffé multiple contrasts for pairwise comparisons. Values in each data set followed by same letter do not differ significantly (p = .05).

[2] pH data anti ln Y transformed prior to analysis. Values presented are ln transformed means.

[3] Values in depth $\overline{X}$ column or peat source $\overline{X}$ row followed by same letter do not differ significantly.

Table 2. Biological characteristics of undisturbed muskeg peat and stockpiled peat (peat stockpiled for 8 months)[1].

Biological parameter	Peat source	Sampling depth (cm)	
		0-15	50-100
Microbial respiration ($\mu l\ CO_2 \uparrow 100\ g^{-1}$ dwt hr^{-1})	Undisturbed	1349^c	198^a
	Stockpile	467^b	414^b
Microbial biomass C (mg C 100 g^{-1} dwt)	Undisturbed	629.6^b	65.5^a
	Stockpile	108.8^a	80.7^a

[1] Data for each parameter analyzed by two-way ANOVA after ln (Y + 1) transformation (MSE = .103 and .159 for respiration and biomass respectively). Differences detected by Scheffé multiple contrasts for pairwise comparison. Means are geometric and where followed by same letter do not differ significantly (p = .05).

Table 3. Characteristics of slender wheatgrass grown in peat from a muskeg bog and peat stockpiled for eight months[1].

		Sampling depth (cm)	
Plant parameter	Peat source	0-15	50-100
Shoot wt (mg dwt plant^{-1})	Undisturbed	94.3^{b}	54.4^{a}
	Stockpile	73.9^{b}	59.7^{a}
Root wt (mg dwt plant^{-1})	Undisturbed	127.9^{b}	96.2^{a}
	Stockpile	97.2^{a}	100.1^{a}
Shoot/root ratio	Undisturbed	0.74 (.23)	0.57 (.09)
	Stockpile	0.77 (.15)	0.60 (.13)

[1] Shoot and root wt data analyzed by Kruskal-Wallis test. Values in each data set followed by the same number are not significantly different (p = .05). Values in brackets are standard deviations.

Table 4. Mycorrhizal infection of slender wheatgrass grown in undisturbed muskeg and stockpiled peat (peat stockpiled for 8 months)[1].

Root parameter	Peat source	Sampling depth (cm) 0-15	 50-100
Total root length (m l^{-1} peat)	Undisturbed	545.0^a	578.6^a
	Stockpile	563.5^a	623.1^a
Mycorrhizal root length (m l^{-1})	Undisturbed	61.5	21.9
	Stockpile	0	23.8
with arbuscules	Undisturbed	19.7 (35.5)	5.3 (7.0)
	Stockpile	0	3.4 (5.1)
with vesicles	Undisturbed	4.4 (8.3)	2.3 (5.1)
	Stockpile	0	2.1 (3.0)
with hyphae	Undisturbed	37.4 (55.3)	14.3 (22.8)
	Stockpile	0	18.3 (26.8)
Percent infection	Undisturbed	14.5^a	4.8^a
	Stockpile	0^a	3.1^a

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[1] Where possible, data were analyzed by two-way ANOVA (MSE = 322.82 and 117.05 for total root length and percent infection respectively). Values for each data set followed by the same letter do not differ significantly ($p = .05$). Values in brackets are standard deviations.

Labrador tea or Vaccinium sp. as ground cover were found not to be infected with VAM fungi. No VAM infection was observed in plants grown in the 0-15 cm depth of peat from the stockpile.

Most of the undisturbed 0-15 cm peat samples sieved for VAM spores were found to contain very few spores. Those spores which were observed were in very poor condition and lacked cell contents. They were small (50-80 μm diameter), thick-walled (up to 8 μm thick) and dark brown in color. Attachment points were missing or obscure. Because these spores were in such poor shape they could not be identified with certainty; however, they appeared to be related to the Glomus fasciculatum-aggregatum complex of VAM fungi. Another endophyte, which is distinguished by the production of very fine (2-5 μm wide) mycelium within the root, was observed in roots of slender wheatgrass grown in the stockpiled peat (50-100 cm). This fungus is often referred to as "fine endophyte" or Glomus tenuis (Greenall) Hall (Mosse, 1981).

7.4.2 Effect of Stockpiling Peat for 6 Years on Mycorrhizal Infection in Slender Wheatgrass

Shoot and root weights of slender wheatgrass grown in peat which had been stockpiled for 6 years and subsequently spread on a tailings sand dyke are given in Table 1. Shoot and root production was significantly improved by the addition of fertilizer with a resultant increase in the shoot/root ratio. Interestingly, shoot and root weights produced by slender wheatgrass in the peat stockpiled for 6 years (Table 5) were very similar to those produced by grass grown in peat stockpiled for 8 months (Table 3). The shoot/root ratio of the grass was 0.68 in both the 6 year and 8 month stockpiled peat.

The total length of slender wheatgrass roots was also significantly increased by fertilization (Table 6). Mycorrhizal infection, however, was not influenced by the presence of fertilizer at the rate used in this experiment. A comparison of the percent infection obtained for plants grown in peat stockpiled for 8 month (Table 4) with infection in plants grown in peat stockpiled for 6 year

Table 5. Root and shoot production by slender wheatgrass in fertilized and unfertilized dyke peat. Peat had been stockpiled for 6 years prior to spreading on dyke[1].

Treatment	Shoot wt (mg plant^{-1})	Root wt (mg plant^{-1})	Shoot/Root
Unfertilized	54.8^{a} (12.5)	80.6^{a} (7.3)	0.68
Fertilized	144.2^{b} (10.2)	138.8^{b} (9.3)	1.04

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[1] Data analyzed by Hotelling's T^2 test. Means in each column not followed by the same letter differ significantly ($p = .05$). Values in brackets are standard deviations.

Table 6. Root length and VAM status of slender wheatgrass grown in fertilized and unfertilized dyke peat. Peat had been stockpiled for 6 years prior to spreading on dyke[1].

	Treatment	
	Unfertilized	Fertilized
Total root length (m l^{-1} peat)	325.7^a	507.9^b
Mycorrhizal root length (m l^{-1} peat)	45.8^a	71.7^a
	(23.2)	(74.2)
with arbuscules	18.6^a	26.8^a
	(15.6)	(14.6)
with vesicles	3.0^a	3.7^a
	(1.3)	(4.4)
with hyphae	24.2^a	52.4^a
	(10.2)	(47.6)
with fine endophyte	5.4^a	1.2^a
	(6.5)	(2.6)
Percent infection	13.7^a	16.4^a
	(5.9)	(13.9)

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[1] Hotelling's T^2 test applied to total root and mycorrhizal root length data. Wilcoxon test applied to vesicle and nyphae data and Student t-test applied to other parameters. Means in each row followed by the same letter do not differ significantly ($p \leq .05$). Standard deviations of the means are in brackets.

(Table 6), indicates that there was an increase in VAM inoculum levels from 3% to 14%. The "fine endophyte" mentioned previously was regularly observed in the 6 year old stockpiled peat, particularly in the unfertilized treatment.

7.4.3 Growth of Slender Wheatgrass in 8 Month Old Stockpiled Peat Mixed with Vesicular-Arbuscular Mycorrhizal Fungal Inoculum

The shoot and root weights of 4 and 10 week old slender wheatgrass grown in the presence or absence of G. aggregatum inoculum under fertilized and unfertilized conditions are presented in Table 7. As expected, fertilization greatly improved plant production over the term of the experiment. Over the first 4 weeks of growth the presence of mycorrhizal infection did not significantly alter root and shoot production by slender wheatgrass whether the plants were fertilized or not. However, after 10 weeks of growth, the plants in the unfertilized, inoculated peat were larger and heavier than the unfertilized, uninoculated plants. In contrast shoot production by plants in the fertilized peat was not significantly affected by VAM infection although roots were heavier in the inoculated treatment than in the uninoculated treatment. Mycorrhizal infection was observed in all inoculated treatments at both sampling times.

7.5 CONCLUSIONS AND DISCUSSION

The reduced level of organic matter in the peat stockpile compared with levels in the undisturbed bog may have been due to the incorporation of soil underlying the peat during the stripping operation. Contamination of the muskeg peat with underlying mineral soil may also have caused the decrease in moisture retention capacity observed for the stockpiled peat. The higher pH measured in the stockpiled peat could have been the result of mixing alkaline subsurface mineral soil into the peat or possibly a consequence of drainage and oxidation of the peat. The increase in pH caused by stockpiling could have deleterious effects on availability of inorganic P for plant growth since pH levels over 6.5 can result in

Table 7. Shoot and root production by slender wheatgrass grown in stockpiled peat (50-100 cm) inoculated with *Glomus aggregatum* and fertilized or left unfertilized[1].

Plant parameter		4 week old plants		10 week old plants	
		Fertilized	Unfertilized	Fertilized	Unfertilized
Shoot wt	Inoculated	22^{c}	8^{ab}	132^{d}	22^{c}
(mg dwt plant^{-1})	Uninoculated	14^{bc}	7^{a}	113^{d}	12^{ab}
Root wt	Inoculated	22^{b}	10^{a}	194^{d}	30^{b}
(mg dwt plant^{-1})	Uninoculated	18^{b}	10^{a}	146^{c}	11^{a}

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[1] Data for each parameter analyzed by three-way ANOVA (MSE = .0715 and .1077 for shoots and roots respectively). A three-way interaction was observed for shoot data, hence Scheffé multiple contrasts were applied to individual treatment means. No three-way interaction was observed for root data, hence Scheffé multiple contrasts were applied to two-way means. Values within each data set followed by the same letter(s) do not differ significantly ($p = 0.05$). Data required ln $(Y + 1)$ transformation, so all means are geometric.

the formation of insoluble calcium salts or highly unavailable apatites (Brady, 1974). These reactions would be particularly serious in peaty soils where available P tends to be low. Also the P-fixing power of alkaline peat would be expected to be high, hence this type of peat would require more P fertilizer to correct P deficiencies than a slightly acidic peat would.

The microbial respiration and biomass C in the undisturbed 0-15 cm peat were substantially higher than those measured in peat from the other three treatments. The dense wefts of fungal mycelium observed in the 0-15 cm deep undisturbed peat and the fact that roots with their mycorrhizal and rhizosphere associations were particularly dense at this depth may account for the high biological activity measured in the undisturbed surface peat. Anaerobic conditions and the highly recalcitrant quality of the C in the microbial food base at the 50-100 cm depth in the undisturbed bog are possible explanations for the much reduced microbial activity observed in the deeper bog samples. In comparison with the biological activity in the 0-15 cm undisturbed peat, microbial respiration and biomass decreased substantially after stockpiling - a result which may have been due to the destruction of the root mat, the mixing of the less active, deeper peat with the highly active surface peat, or the dilution of the underlying mineral soil with the peat above it. The microbial biomass in the stockpiled peat was not significantly different from that in the undisturbed 50-100 cm deep peat, suggesting that the peat stockpile is composed mainly of peat in the same state of decomposition as that found in the deep undisturbed peat.

Shoot and root production by slender wheatgrass was poor in all four peat treatments with the best growth occurring in the surface peat from the bog and stockpile. Plants were stunted and exhibited no tillering or seed production over the term of the study. Shoot/root ratios were low emphasizing the need for supplemental nutrients to promote plant growth. The addition of both N and P to a nutrient-deficient soil has been demonstrated to increase shoot/root ratios (see review by Boote, 1977). The apparent P deficiency observed in the slender wheatgrass grown in the 50-100 cm deep stockpiled peat may have been partially due to the high pH (7.7) of this peat rendering much of available P insoluble.

The low available P status characteristic of peaty soils (Brady, 1974), particularly those with a high pH, would suggest that the mycorrhizal symbiosis would be essential to the growth and maintenance of plants used to revegetate tailings dykes amended with peat. In situations where fertilization of peat amended tailings sand is kept to a minimum or in revegetated areas where maintenance fertilizer applications are halted, the presence of the mycorrhizal association may be crucial to the success and survival of the established plant cover.

In view of the fact that many of the plant species used in the revegetation of the oil sands are species whose roots are commonly infected by vesicular-arbuscular mycorrhizal (VAM) fungi, VAM fungal inoculum should be present in the amendments applied during the reclamation process. However, both the undisturbed and stockpiled peat used in the present study contained very low levels of VAM inoculum. This is not surprising since the vegetation on the peat bogs in the oil sands region is often dominated by non-VA hosts such as tamarack, black spruce and ericaceous plants. Both tamarack and black spruce are hosts for ectomycorrhizae while the Ericales form arbutoid or ericoid type mycorrhizae. Inter- and intracellular penetration of the root cortex by septate hyphae occurs in the ericaceous hosts, but no vesicles or arbuscules are formed. The fungi involved in the formation of ectomycorrhizae and arbutoid or ericoid-type mycorrhizae are taxonomically very different from those which form VA mycorrhizae.

Although many of the plant species in the peat bog in this study were non-VA hosts, VA inoculum did occur in areas where VA hosts (grasses) were present. However, compared with infection levels found in grasslands (approx. 60%), the infectivity potential of the peat, even when originally occupied by a VA host, was low. VA inoculum was also present in the deeper, undisturbed peat and stockpiled peat but at levels which produced very low infection. The very low VAM infection potential of peat from northern Alberta, in particular peat being used in revegetation programs, has been observed in other studies (Visser et al., 1981; Danielson et al., 1983).

Since VAM inoculum is present in the peat when stockpiled, it might be assumed that by planting a VA host an increase in inoculum would result. When comparing percent VAM infection in slender wheatgrass grown in peat stockpiled and unvegetated for 8 mo and peat stockpiled and vegetated with grass for 6 years, a 10-12% increase in VAM infection was observed over the stockpiling period. The very low increase in infection indicates that VA inoculum levels build up very slowly in the peat stockpile, perhaps due to the inefficient dispersal of the VA spores or the restricted rate of growth of the fungus within the host root system resulting from excessive fertilization of the stockpile. Although low rates of fertilizer did not appear to inhibit infection in grass grown in the 6 year old stockpiled peat in this study, there are many studies which have demonstrated that high fertilizer applications (particularly N and P) will inhibit mycorrhizal formation (see review by Hayman, 1982). It should also be kept in mind that the rooting zone of a revegetated stockpile is concentrated in the surface 15 cm. Any VA inoculum accumulation in this zone will eventually be diluted by the deeper, inoculum-free peat when the peat is spread on the tailings sand dyke.

The importance of the mycorrhizal symbiosis in stimulating plant growth in the mycorrhizal and P deficient peat stockpiled for 8 months was evidenced in greater shoot and root production by slender wheatgrass when a VAM fungus was artificially introduced to the peat. Some of the P deficiency symptoms (deep green to purple leaves) noted for plants grown in this peat also appeared to be partially relieved by VAM infection. Although mycorrhizal infection stimulated plant production, 10 week old plants were still extremely small. Addition of low doses of fertilizer substantially improved shoot production, but in the presence of fertilizer, VAM infection did not have a significant effect on shoot weights. These data indicate that for fibrous rooted species such as grasses, fertilization may override the effects of VAM infection on shoot production. More research is required to determine at which fertilizer levels VAM infection is most beneficial to the host. No doubt this will vary with the host.

In summary this study has shown that both undisturbed bog peat and stockpiled peat on the Syncrude site appear to have negligible quantities of VAM inoculum. Revegetating peat stockpiles with VAM hosts (e.g. grasses, legumes) will increase the infectivity potential of the peat, but this increase seems to occur at a very slow rate. The lack of VAM inoculum in the peat used to revegetate tailings dykes should be considered when planning a reclamation program. The dependence of a plant on the VAM symbiosis will vary with its demand for P and the efficiency with which the plant obtains P from the soil. Hayman (1982) generalized that fibrous rooted species where soil-root contact is high (e.g. crop plants) are less likely to respond to VAM infection than plants with coarser root systems. Many of the shrub species being considered for reclamation of the tailings dyke may have much coarser root systems than the grasses and therefore may be highly dependent on the mycorrhizae for their survival. These species may require the introduction of inoculum. Mosse (1981) stated that "soil infectivity will be reduced in sites long inhabited by non-host plants and that such sites may be difficult to colonize subsequently with highly mycorrhiza-dependent plants". The undisturbed peat bogs in the oil sands region are good examples of such sites.

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8. EFFECTS OF THREE LEVELS OF FERTILIZER AND INOCULATION ON THE GROWTH AND MYCORRHIZAL DEVELOPMENT OF CONTAINERIZED JACK PINE SEEDLINGS

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8.1 ABSTRACT

Jack pine seedlings were grown in 65 cm^3 containers filled with a peat:vermiculite mixture or pure peat. Seedlings were fertilized twice weekly for 20 weeks with complete fertilizer solutions containing either 15, 30 or 60 mg N l^{-1}. Differences in plant growth and mycorrhizal formation were minimal between the two growing media. The weight of roots was not affected by fertilizer levels but the high level of fertilizer reduced the total number of short roots produced. Shoot growth was increased by using 30 mg N/l but not further increased by using 60 mg N l^{-1}. Inoculation with E-strain reduced shoot growth 35%. Infection by E-strain exceeded 95% in all treatments, whereas infection by *Laccaria proxima* was reduced when 60 mg N l^{-1} was used. Results with *Pisolithus tinctorius* were highly variable but some infection occurred in all treatments. Mycorrhizae formed by *Sphaerosporella brunnea* occurred only when the lowest amount of fertilizer was applied.

8.2 INTRODUCTION

Contemporary objectives in the production of containerized seedlings intended for reforestation not only include the production of seedlings with favorable shoot and root sizes but also root systems infected with mycorrhizal fungi (Marx *et al*., 1982). These two objectives may be counter-productive as the use of high levels of fertilizer needed to produce large seedlings may preclude mycorrhizal infection (Bjorkman, 1970). Conversely, the use of low levels of fertilizer may allow mycorrhizal development but result in seedlings well below the size expected and desired by forest managers. Currently it is felt that sacrificing size to permit mycorrhizal development may result in seedlings better adapted to cope with the stresses encountered in field sites (Marx *et al*., 1982; Molina, 1979). The benefits of inoculating seedlings with specific fungi have been amply demonstrated by the numerous outplantings utilizing *Pisolithus tinctorius* (Pers.) Coker & Couch (e.g. Marx, 1980). Although *Pisolithus* readily forms mycorrhizae under greenhouse conditions, the amount of fertilizer or different combinations of nutrient elements that allow satisfactory mycorrhizal development by most other fungal symbionts is largely unknown. At present, only *Pisolithus tinctorius* has been tested in fertilizer trials with containerized seedlings. The situation is further complicated by the great variety of fertilizer formulations, rates of applications, timing of applications, solubilities, container size and growing media that have been used (see Appendix Tables 4 and 5), thus precluding comparisons among studies. As suggested by Molina (1980), greenhouse fertilization trials are required to determine the limits of nutrients that will permit establishment of mycorrhizae of container-grown seedlings. No doubt, numerous trials will be necessary before predictive generalizations will be possible on the multitude of host-symbiont combinations.

The major objective of this study was to determine the effect of three levels of fertilizer on the growth of containerized jack pine seedlings and the degree of mycorrhizal development induced by four symbionts. A secondary objective was to determine if a mixture of peat and vermiculite as compared to pure peat produced different growth responses of jack pine or affected mycorrhizal infection. Information obtained from this preliminary study will be applied to more detailed studies of containerized jack pine seedlings and the refinement of inoculation techniques with the long range objective of utilizing mycorrhizal seedlings in the forestation of sites exhibiting severe nutrient and water stresses.

8.3. MATERIALS AND METHODS

The fungi tested were Laccaria proxima Boudier, Sphaerosporella brunnea (Alb. & Schw. ex Fr.) Svrcek & Kubicka, Pisolithus tinctorius (Pers.) Coker & Couch (Marx isolate 185) and the E-strain. Inoculum was prepared in respiration tubes which allowed the activity of the fungi to be constantly monitored (Visser and Danielson, unpubl. data). These tubes were also used to determine the effects of autoclaving the peat-vermiculite substrate on subsequent activity of mycorrhizal fungi (Visser and Danielson, unpubl. data). The tubes contained peat (< 2 mm fraction) and vermiculite (> 2 mm fraction) in proportions of 1:15 (v/v) and was moistened with modified Melin Norkran's (MMN) solution (Marx and Bryan, 1965) and autoclaved for 15 min. Each tube was inoculated with a mycelial slurry prepared by homogenizing colonies from MMN agar plates in a Virtis homogenizer. At the end of a 3 mo incubation period the inoculum was checked for viability and contamination by plating particles on potato dextrose agar and the inoculum washed in cold tap water (Marx and Bryan, 1975). The washed inoculum was incorporated at a rate of 1:9 (v/v) with either pure commercial peat or a 1:1 mixture of peat and vermiculite. The planting mixture was packed into 65 cm^3 Leach Cone-tainers (Ray Leach, Canby, Or.) and each cell planted with a surface sterilized, pregerminated jack pine seed. The peat used in the planting mixture had been autoclaved several months previously and inoculated with a soil suspension of a prairie soil free of ectomycorrhizal symbionts. Ten replicate cells were prepared for each treatment.

The seedlings were grown in the greenhouse with an extended photoperiod using Glo-lux lights giving a minimum of 3.5 klx. During first 11 wk the photoperiod was 15 h and was extended to 18 h for the remainder of the trial period.

For the first two weeks after planting no fertilizer was applied. During the third week a solution containing 160 mg l^{-1} of 10:42:10 was applied twice to all treatments. The fertilizer regimes used for weeks 4-20 consisted of either 100, 200 or 400 mg l^{-1} of 15:15:18 Plant Prod Soilless feed with Fe EDTA and micronutrients. All containers were fertilized twice a week and watered once a week until the solutions dripped freely through the drain holes in the tubes.

Five replicates of each treatment were harvested at 13 wk and weights of shoots and roots determined after drying at 80°C. The entire root system was cut into 2-3 cm segments and randomly selected until 300 short roots had been evaluated for mycorrhizal infection. The short roots were rated using 12-25X magnification. High resolution examinations of whole mounts using 500X brightfield optics were done to detect early stages of infection or poorly differentiated mycorrhizae. When the seedlings were 20 wk old the evaluations were repeated with the exception that Laccaria proxima was eliminated after 13 wk to prevent basidiospore contamination of the other treatments. Infection by E-strain was not evaluated at 20 wk as maximum mycorrhizal development occurred by 13 wk. Total short roots per seedling were calculated by weighing the sample with 300 short roots and the uncounted sample separately.

8.4 RESULTS

At the end of 20 wk there were no differences in shoot growth between plants grown in pure peat and those grown in the peat-vermiculite mixture and fertilized with the low nutrient regime (Table 1). At 13 wk, shoots in the 200 and 400 mg l^{-1} treatments and inoculated with E-strain or uninoculated were significantly larger than those in the 100 mg l^{-1} treatment. When 20 wk old, shoots in the 100 mg l^{-1} treatment were significantly smaller

Table 1. Effects of symbionts and fertilizer levels on shoot growth of container-grown jack pine seedlings.

		Peat:Vermiculite (1:1)			Peat
		Fertilizer level (mg l^{-1})			
Symbiont	Age (wk)	100	200	400	100
		Shoot weight (mg)			
Laccaria proxima	13	102^{a}	139^{a}	149^{a}	ND
Sphaerosporella	13	113^{a}	164^{a}	136^{a}	ND
brunnea	20	289^{a}	471^{b}	551^{c}	ND
Pisolithus	13	93^{ab}	129^{ab}	153^{b}	87^{a}
tinctorius	20	265^{a}	401^{b}	496^{b}	282^{a}
E-strain	13	77^{a}	125^{b}	125^{b}	112^{b}
	20	203^{a}	347^{b}	385^{b}	198^{a}
Control	13	100^{a}	136^{c}	121^{bc}	100^{ab}
	20	362^{a}	528^{b}	544^{b}	328^{a}

Data for 13 wk old seedlings inoculated with the four fungi analyzed by one-way ANOVA and differences among means tested by Scheffé pairwise comparisons. Control 13 wk seedlings all 20 wk old analyzed by one-way MANOVA. Values in each row followed by the same letter do not differ significantly ($p = .05$).

ND = not determined.

than in the two higher fertilization treatments regardless of inoculum treatment. There was no difference in shoot sizes between 200 and 400 mg l^{-1} rates except with S. brunnea. Seedlings inoculated with E-strain were consistently smaller than those not inoculated or inoculated with the other fungi.

Root growth was not affected by the type of growing medium (Table 2). At 13 wk in the control, root weight was significantly less at the high nutrient level than when less fertilizer was used. A similar trend occurred with seedlings inoculated with S. brunnea. At 20 wk, root weights were unaffected by fertilizer concentration except in the S. brunnea treatment. Inoculation with E-strain resulted in reduced shoot growth at all fertilizer levels (Table 3). Although shoots of mycorrhizal plants weighed only 64% as much as shoots in the control treatment, root growth was not significantly affected.

Shoot:root ratios decreased markedly when more than 100 mg l^{-1} of fertilizer was applied (Table 4). The trend was for shoot:root ratios to decrease as the seedlings aged.

At twenty weeks, total plant weight at 400 mg l^{-1} varied from 740 to 809 mg. At 200 mg l^{-1} weights varied from 612 to 788 mg and at 100 mg l^{-1} mean seedling weights ranged from 495 to 625 mg.

Infection by E-strain was very high in all treatments after 13 wk (Table 5). Mycorrhizal development did not appear to be influenced by the type of growing medium although only two fungi were tested in the peat substrate. The results from inoculation with Pisolithus were too variable to allow confident conclusions to be made on either the effects of growing medium or fertilization on infection. Both L. proxima and E-strain infected significantly less short roots when fertilized with 400 mg l^{-1} than with 100 or 200 mg l^{-1} but levels of infection under the high nutrient regime were substantial. S. brunnea was more sensitive than the other fungi to the effects of fertilizer additions as no infections were detected above the lowest rate applied. The percentage of short roots infected by S. brunnea and Pisolithus failed to increase after 13 wk

Table 2. Effects of symbionts and fertilizer levels on root growth of container-grown jack pine seedlings.

Symbiont	Age (wk)	Peat:Vermiculite (1:1) Fertilizer level (mg l^{-1}) 100	Peat:Vermiculite (1:1) Fertilizer level (mg l^{-1}) 200	Peat:Vermiculite (1:1) Fertilizer level (mg l^{-1}) 400	Peat Fertilizer level (mg l^{-1}) 100
		Root weight (mg)			
Laccaria proxima	13	78^a	73^a	63^a	ND
Sphaerosporella	13	78^{ab}	83^b	54^a	ND
brunnea	20	231^a	278^b	258^b	ND
Pisolithus	13	66^a	71^a	56^a	47^a
tinctorius	20	230^a	211^a	247^a	203^a
E-strain	13	77^{ab}	68^{ab}	46^a	96^b
	20	189^a	188^a	219^a	202^a
Control	13	98^b	85^b	57^a	110^b
	20	263^a	260^a	252^a	244^a

Data for 13 wk old seedlings inoculated with the four fungi analyzed by one-way ANOVA and differences tested by Scheffé pairwise comparisons. Control data and all 20 wk data analyzed by one-way MANOVA. Values in each row followed by the same letter do not differ significantly ($p = .05$).

ND = not determined.

Table 3. Effects of inoculation with the E-strain on shoot and root growth after 20 wk using three fertilizer regimes.

	Peat:Vermiculite (1:1)			Peat	
	Fertilizer level (mg l^{-1})				
	100	200	400	100	
Treatment	Root weight (mg)				Mean
Control	263	260	252	244	255^{a}
E-strain	189	188	219	202	200^{a}
mean	226^{a}	224^{a}	236^{a}	223^{a}	
	Shoot weight (mg)				
Control	362	528	544	328	441^{b}
E-strain	203	347	385	198	283^{a}
mean	283^{a}	438^{b}	465^{b}	263^{a}	

Data analyzed by two-way MANOVA. Values in each row and column followed by the same letter do not differ significantly ($p = .05$).

Table 4. Shoot:root ratios of jack pine seedlings inoculated and fertilized at three different rates.

		Peat:Vermiculite (1:1)			Peat
		Fertilizer level (mg l^{-1})			
	Age	100	200	400	100
Symbiont	(wk)	Root weight (mg)			
Laccaria laccata	13	1.4	2.0	2.3	ND
Sphaerosporella brunnea	13	1.5	2.0	2.5	ND
	20	1.2	1.7	2.1	ND
Pisolithus tinctorius	13	1.4	1.8	2.7	2.3
	20	1.1	1.9	2.0	1.4
E-strain	13	1.0	2.0	2.4	.9
	20	1.0	1.8	1.7	1.0
Control	13	1.0	1.7	2.2	1.4
	20	1.4	2.0	2.2	1.3

Values are ratios and thus not subject to statistical analyses.

ND = not determined.

Table 5. Effects of symbionts and fertilizer levels on mycorrhizal infection of container-grown jack pine seedlings.

		Peat:Vermiculite (1:1)						Peat	
		Fertilizer level (mg l^{-1})							
		100		200		400		100	
	Age	PERCENT INFECTION							
Symbiont	(wk)	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Laccaria proxima	13	95^a	2	90^b	10	44^a	5	ND	ND
Sphaerosporella brunnea	13	12^b	4	0^a	0	0^a	0	ND	ND
	20	11^b	7	0^a	0	0^a	0	ND	ND
Pisolithus tinctorius	13	41^b	38	2^a	6	1^a	1	7^a	16
	20	22^a	34	0	0	12^a	26	5^a	12
E-strain	13	99^b	.2	99^b	.9	95^a	5	99^b	1
Control	13	0	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0	0

Data analyzed by one-way ANOVA after $2 \arcsin \sqrt{p}$ transformation and differences among means tested by Scheffé pairwise comparisons except for *Sphaerosporella* data where differences based on 95% C.I. for $\bar{x}$. Values in each row followed by the same letter do not differ significantly ($p = .05$).

ND = not determined.

although the total number of infected roots increased substantially (see below).

At 13 wk mycorrhizal infections were visually less obvious at the high fertilizer levels than at the low levels. At the 100 mg l^{-1} fertilization level, infections by E-strain, Pisolithus and Laccaria were obvious without magnification. Only Laccaria resulted in obvious mycorrhizal structures at the 200 mg l^{-1} level. At 400 mg l^{-1}, none of the fungi appeared to form mycorrhizae without more critical observations. In pure peat, although E-strain infected 99% of the short roots when observed microscopically, a casual judgment indicated no infections were present.

Short roots infected with E-strain were predominantly unbranched in all treatments except at the 200 mg l^{-1} fertilization level. Laccaria proxima produced mushroom initials commonly in the low fertilization treatment but only rarely in the two higher levels. As well, branching of mycorrhizal short roots was reduced in the 200 and 400 mg l^{-1} treatments. In general it appeared that increased rates of fertilization depressed mycorrhizal development, fruit body formation and the production of extramatrical mycelium.

The number of short roots produced did not vary with the two planting mixtures except in the control, where the total number was greater in the peat than in the peat-vermiculite mixture (Table 6). Increased fertilization resulted in reduced numbers of short roots in the control and S. brunnea treatments after 20 wk but not with seedlings inoculated with Pisolithus. The number of short roots of S. brunnea and Pisolithus seedlings increased about 3-fold between 13 and 20 wk while the percentage of infection remained relatively constant (see Table 5).

The production of short roots in terms of total root weight was largely unaffected by the growing medium except in the control at 20 wk when there were significantly less short roots in the peat-vermiculite than in the peat (Table 7). At 20 wk, significantly less short roots were produced in the control and S. brunnea treatments when fertilized with 400 mg l^{-1} of 15:15:18 than when a rate of 100 mg l^{-1} of fertilizer was used. Overall, the higher the rate

Table 6. Effect of symbionts and fertilizer levels on numbers of short roots and container-grown jack pine seedlings.

		Peat:Vermiculite (1:1)			Peat
		Fertilizer level (mg l^{-1})			
	Age	100	200	400	100
Symbiont	(wk)	Total short roots/plant			
Laccaria proxima	13	636^{a}	564^{a}	450^{a}	ND
Sphaerosporella brunnea	13	808^{a}	626^{a}	459^{a}	ND
	20	2449^{b}	2225^{ab}	1980^{a}	ND
Pisolithus tinctorius	13	727^{a}	492^{a}	456^{a}	629^{a}
	20	2174^{a}	1804^{a}	1712^{a}	2229^{a}
E-strain	13	670^{ab}	603^{ab}	425^{a}	811^{b}
Control	13	1004^{b}	536^{ab}	188^{a}	644^{ab}
	20	2412^{b}	2712^{bc}	1602^{a}	2992^{c}

Data for 13 wk old seedlings analyzed by one-way ANOVA and differences among means tested by Scheffé pairwise comparisons. Data on 20 wk old seedlings tested by one-way MANOVA. Values in each row followed by the same letter do not differ significantly ($p = .05$). ND = not determined.

Table 7. Effect of symbionts and fertilizer levels on the numbers of roots per unit weight of the root system of container-grown jack pine seedlings.

		Peat:Vermiculite (1:1)			Peat
		Fertilizer level (mg l^{-1})			
	Age	100	200	400	100
Symbiont	(wk)	Short roots/mg weight			
Laccaria proxima	13	8.3^{a}	8.4^{a}	7.4^{a}	ND
Sphaerosporella	13	10.4^{a}	7.7^{a}	8.8^{a}	ND
brunnea	20	10.6^{b}	8.2^{a}	7.9^{a}	ND
Pisolithus	13	10.9^{ab}	6.9^{a}	8.0^{a}	13.4^{b}
tinctorius	20	9.7^{a}	8.8^{a}	7.0^{a}	12.7^{a}
E-strain	13	9.0^{a}	8.6^{a}	9.7^{a}	8.6^{a}
Control	13	10.1^{b}	6.5^{ab}	3.6^{a}	8.2^{b}
	20	9.1^{b}	10.5^{b}	6.4^{a}	12.5^{c}

Data for 13 wk old seedlings analyzed by one-way ANOVA and differences among means tested by Scheffé pairwise comparisons. Data for 20 wk old seedlings tested by one-way MANOVA. Values in each row followed by the same letter do not differ significantly ($p = .05$). ND = not determined.

of fertilizer, the less short roots that were produced per unit of total root weight.

8.5 DISCUSSION

Although it has been suggested that pure peat is superior to a mixture of peat and vermiculite for plant growth (Carlson, 1979) the results of this study indicates that the growing medium did not substantially affect mycorrhizal formation or plant growth. The comparison in this study was conducted under a low nutrient regime but it is unlikely that results would be otherwise with higher levels of nutrients. For mycorrhizal evaluations, root systems are easier to clean with vermiculite included in the growing medium than with pure peat, thus offering an experimental advantage for using peat-vermiculite.

Although generalizations on the effects of nutrient levels upon mycorrhizal formation have been developed, there appears to be sparce data to support the concensus that reasonable, operational levels of nutrients suppress mycorrhizal formation. In fact, the only mycorrhizal fungus to be subjected to extensive testing under varying levels of nutrients, Pisolithus tinctorius, has formed mycorrhizae under all the conditions it has been subjected to (Beckford et al., 1980; Dixon et al., 1981; Marx and Barnett, 1974; Maronek et al., 1981; Ruehle, 1980; Ruehle and Marx, 1977). This should not suggest a generality for all fungi however; Pisolithus tinctorius occupies a taxonomic position, and possibly an ecological position, far distant from the majority of other mycorrhizal fungi. It would not seem unlikely that many members of the Agaricales (sensu lato) and the Aphyllophoralles might well behave differently and tolerate broader or require narrower nutrient regimes.

Inoculation either did not affect seedling growth or caused a reduction in size. Other studies of container-grown seedlings have reported growth enhancement by mycorrhizae (Maronek et al., 1981), a reduction in growth (Shaw et al., 1982) or no effect (Marx et al., 1982). No effect on growth or a depression is to be expected as root density is very high in small containers with the entire volume being thoroughly exploited by the roots. As diffusion to the roots is not limiting, mycelial development does not benefit either nutrient or water uptake. The only effect of the fungus is probably to produce a C drain on the plant and reduce plant growth. In cases where there is a growth stimulation it could be due to the use of a large container in which the roots do not effectively colonize the growing mixture and mycelium is required to fully exploit the growing medium for low mobility nutrients. This phenomenon may also function in closed synthesis flasks when root systems are very small (Alexander, 1981). However in flasks, transpiration does not occur and thus mass flow to the roots is negligible. In addition, the results obtained by Alexander (1981) were very reminiscent to those of Zak (1971) who attributed growth differences to detoxication of autoclaved soil by Corticium bicolor Peck rather than enhanced nutrient uptake. Any instances of significant stimulations of growth in containers caused by mycorrhizal infection should be critically examined to determine if nonnutrient effects are responsible as these may suggest functional differences among mycorrhizal fungi.

Both Laccaria and E-strain formed adequate levels of mycorrhizae even when 60 mg N 1^{-1} was being applied. Operational levels of fertilizer for jack pine vary from 229 mg N 1^{-1} once per week (Carlson, 1979) to 100 mg N 1^{-1} twice per week (B. Fessenden, personal comm.). Mycorrhizae have been reported to form with western hemlock and Cenococcum, Piloderma croceum (Bres.) Erikss. & Hjortz. and Thelephora terrestris under operational fertilizer regimes utilizing 100 mg N 1^{-1} applied twice weekly (Kropp, 1982). Other reports of mycorrhizae formation are impossible to compare due to the different methods of fertilizer application. However it is apparent that some fungi can form mycorrhizae with some hosts under relatively high nutrient regimes. Additional studies are required to determine the effects of frequency of application as well as nutrient concentrations on mycorrhizal formation. Most importantly a wide variety of fungi should be tested in order to determine if infectivity varies among ecological, taxonomic or morphological groups with regard to fertilization levels.

8.6 LITERATURE CITED

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9. SUMMARY AND OUTLOOK

The seedlings used in the outplanting study were small in comparison to those produced for operational forestation programs. However, it is now possible to obtain considerably larger seedlings by increasing fertilization rates without diminishing the mycorrhizal status when aggressive symbionts are used. Prior to initiating additional performance trials, the fertilizer tolerance of prospective symbionts should be evaluated so that preplant seedling size can be maximized. When nutrient sensitive symbionts are used, different fertilization patterns may be required to produce plantable seedlings.

During the first season's growth, inoculation had no significant effect on plant growth. The amount of root growth into the amended tailings sand was small and thus most water and nutrient uptake probably occurred in the volume occupied by the planting plug which contained a high density of roots. This would serve to minimize any potential effect of mycorrhizal status. In that the growing season is so short, it may take several years for treatment effects to be clearly expressed. The aggressive colonization of extending roots by four of the fungi should provide a basis for evaluating performances of both host and symbionts in the second growing season. It would be expected that these fungi will persist on the root systems as three of the four, Thelephora, Laccaria and Hebeloma were isolated from northern Alberta, and the fourth, the E-strain, has persisted on jack pine for over four years in amended tailing sand.

Of considerable importance will be the development of mycorrhizae by fungi indigenous to the peat and the stability of the associations. Even though stockpiling reduced inoculum levels of ectomycorrhizal fungi, it can be expected that a significant portion of the root systems will become infected with indigenous species. If these species were to outcompete the introduced species for infection sites, inoculations would be rendered ineffectual. Continued observations should indicate the competitive abilities of the indigenous fungi although inoculation trials may be necessary to fully assess this factor.

In contrast to the ectomycorrhizal inoculum, stockpiling resulted in apparent increases in VA inoculum due to the introduction of VA host plants to stabilize the stockpile. Nonetheless, infection levels of VA plants in the amended tailings sand was low and thus of questionable benefit to the host. Now that it has been established that inputs of VA inoculum to tailings sand can be anticipated to be low, further attention should be paid to the fate of that inoculum with regard to fertilization practices so that levels are not reduced even further and make recovery more difficult once nutrients are withheld. In that actinomycete-nodulated nitrogen-fixing shrubs may be largely dependent on VAM or ectomycorrhizae for nutrients other than nitrogen, special attention should be given to the mycorrhizal relationships of these plants. It is to be expected that these shrubs, especially alder, are more specialized with regard to their fungal associates and thus the indigenous inoculum factor becomes more critical than with either jack pine or slender wheatgrass. It is also possible that some of these shrubs may be more dependent upon the mycorrhizal state than pine or grasses and this factor should be evaluated. It may be of less than academic interest to use these often pioneering plants to test the relative benefits of VA versus ectomycorrhizal associations with regard to plant performance and nitrogen inputs into the soil.

Based on field and greenhouse studies a picture is emerging of the mycorrhizal potential of peat overburden, the problems involved in manipulating the mycorrhizae and which host plants should be emphasized. Future work should concentrate on natural associations of key host species, stability of spontaneous and induced associations and the improvement of techniques for enhancing desirable symbiotic relationships.

10. APPENDICES

Appendix Table 1. Experimental design and first year sampling pattern of the mycorrhizal jack pine outplanting study.

This file contains the sampling grid for the Syncrude dyke jack pine outplanting experiment. Each column of the grid represents a row of trees similarly inoculated. Columns 1 - 27 are as follows:

Column	Treatment	Planters	Date planted (1982)
1	Amphinema byssoides	SV & RMD	June 16
2	Syncrude stock	K or M	June 17
3	Control	SV & RMD	June 16
4	Syncrude stock	K or M	June 17
5	Hydnum imbricatum	SV & RMD	June 16
6	Tricholoma flavovirens	V & RMD	June 16
7	Lactarius paradoxus	RMD & JC	June 16
8	Astraeus hygrometricus	RMD & JC	June 16
9	Hydnum imbricatum	SV & RMD	June 16
10	Laccaria proxima	K or M	June 16
11	Pisolithus tinctorius	K or M	June 17
12	Thelephora terrestris	K or M	June 17
12	Thelephora terrestris	K or M	June 17
14	F-strain	SV & RMD	June 17
15	Hebeloma sp.	SV & RMD	June 17
16	Hebeloma sp.	SV, RMD &BF	June 16
17	Astraeus hygrometricus	SV & RMD	June 17
18	Cenococcum geophilum	SV & RMD	June 17
19	Sphaerosporella brunnea	SV & RMD	June 17
20	Pisolithus tinctorius	K or M	June 17
21	Lactarius paradoxus	RMD & JC	June 16
22	Laccaria proxima	K or M	June 17
23	E-strain	SV & RMD	June 17
24	Sphaerosporella brunnea	K or M	June 17
25	Amphinema byssoides	SV & RMD	June 16
26	Control	SV & BF	June 16
27	Cenococcum geophilum	SV & RMD	June 17

Lines are 1 m apart up the dyke, seedlings .5 m apart in each line
SV = S. Visser, RMD = R.M. Danielson, BF = B. Fessenden, JC = J. Campbell, K = Kim McCumber, M = Monica
Surface 5 cm dry on June 16, shower in the evening which resulted in good moisture levels to the surface on June 17.
Codes for grid numbers (code is immediately to the right of seedling number)
**1 - seedling sampled in September 1982
$$$ - alternate sample
NNN - never planted
DD1 - dead as of September 1982
In September 1982 5 evenly numbered seedlings were randomly selected from each column for sampling

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38  76 **1 152 190 228 266 304 NNN 380 418 456 NNN **1 **1 NNN NNN 684 722 760 NNN NNN NNN NNN NNN NNN NNNN

37  75 113 151 189 227 265 303 341 379 417 455 NNN 531 569 NNN NNN 683 721 759 797 NNN NNN NNN NNN NNN NNNN

36  74 112 150 **1 **1 **1 **1 340 378 **1 454 **1 **1 **1 606 NNN **1 720 758 **1 834 **1 **1 948 986 ****

35  73 111 149 187 225 263 301 339 377 415 453 491 523 567 605 642 681 719 757 795 833 871 909 947 985 1023

**1  72 110 148 186 224 **1 300 **1 376 414 452 490 528 566 604 642 680 718 756 **1 832 870 908 946 984 1022

33  71 109 147 185 223 261 299 337 375 413 451 489 527 565 603 641 679 717 755 793 831 869 907 945 983 1021

**1 **1 108 **1 184 **1 260 298 336 **1 **1 **1 488 526 564 **1 **1 **1 716 754 792 **1 868 906 944 **1 1020

31  69 107 145 183 221 259 297 335 373 411 449 487 525 563 601 639 677 715 753 791 829 867 905 943 981 1019

30  68 106 144 **1 220 258 296 334 372 410 **1 486 524 **1 600 638 676 714 752 790 828 **1 904 **1 980 1018

29  67 105 143 181 219 257 295 333 371 409 447 485 523 561 599 637 675 713 751 789 827 865 903 941 979 1017

28  66 **1 142 **1 218 256 294 **1 370 408 446 484 522 560 598 **1 674 **1 **1 788 826 864 902 940 978 1016

27  65 103 141 179 217 255 293 331 369 407 445 483 521 559 597 DD1 673 DD1 749 787 825 863 901 939 977 1015

26 **1 102 140 178 216 254 292 DD1 368 406 444 482 520 558 596 634 672 710 748 **1 **1 862 900 **1 976 1014

25  63 101 139 177 215 253 291 329 367 405 443 481 519 557 595 633 671 709 747 785 823 861 899 937 975 1013

24  62 **1 138 176 214 252 290 328 366 **1 442 **1 518 556 594 631 670 708 **1 784 822 **1 898 **1 974 ***1

23  61  99 137 175 213 251 289 327 365 403 441 479 517 555 593 631 669 707 745 783 821 859 897 935 973 1011

22  60 **1 136 174 212 250 288 **1 364 DD1 **1 478 516 554 **1 **1 **1 **1 **1 782 **1 858 896 934 **1 1010
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 21  59  97 135 173 211 249 287 325 363 401 439 477 515 553 591 629 667 705 743 781 819 857 895 933 971 1009
**1 **1  96 **1 172 210 **1 286 324 **1 400 438 476 514 552 590 628 666 704 742 780 818 **1 **1 932 970 1008
 19  57  95 133 171 209 247 285 323 361 399 437 475 513 551 589 627 665 703 741 779 317 855 893 931 969 1007
 18  56  94 **1 **1 208 **1 **1 322 360 398 436 474 512 550 **1 626 664 **1 740 778 **1 854 **1 930 968 1006
 17  55  93 131 169 207 245 283 321 359 397 435 473 511 549 587 625 663 701 739 777 815 853 891 929 967 1005
 16 **1  92 130 168 206 244 282 **1 358 396 434 **1 510 548 586 624 662 700 738 776 814 852 890 **1 **1 1004
 15  53  91 129 167 205 243 281 319 357 395 433 471 509 547 585 623 661 699 737 775 813 851 889 927 965 1003
 14 **1  90 **1 166 204 242 280 318 **1 394 432 **1 **1 546 584 622 660 698 736 **1 **1 850 888 926 964 1002
 13  51  89 127 165 203 241 279 317 355 393 431 469 507 545 583 621 659 697 735 773 811 849 887 925 963 1001
 12  50  88 126 164 202 240 278 316 354 **1 430 468 506 544 **1 **1 658 696 734 772 810 848 **1 924 **1 ***1
 11  49  87 125 163 201 239 277 315 353 391 429 467 505 543 581 619 657 695 733 771 809 847 885 923 961  999
**1  48 **1 124 162 **1 238 276 314 352 390 428 466 **1 **1 580 618 **1 694 **1 **1 808 **1 **1 922 960  **1
  9  47  85 123 161 199 237 275 313 351 389 427 465 503 541 579 617 655 693 731 769 807 845 883 921 959  997
  8  46  84 **1 160 198 236 **1 312 350 388 426 **1 502 540 578 **1 654 **1 DD1 768 806 844 882 **1 **1  **1
  7  45  83 121 159 197 235 273 311 349 387 425 463 501 539 577 615 653 691 729 767 805 843 881 919 957  995
  6  44  82 120 158 **1 **1 272 310 **1 386 424 462 500 538 576 614 **1 690 **1 766 804 842 880 918 956 994
  5  43  81 119 157 195 233 271 309 347 385 423 461 499 537 575 613 651 689 727 765 803 841 879 917 955  993
  4  42  80 118 156 **1 232 **1 **1 346 384 **1 450 498 **1 **1 612 650 688 726 764 802 840 878 916 954  992

  3  41  79 117 155 193 231 269 307 345 383 421 459 497 535 573 611 649 687 725 763 801 839 877 915 953  991

**1  40  78 116 **1 192 230 **1 306 **1 **1 **1 458 **1 534 572 610 648 **1 724 762 800 838 876 914 952  990

  1  39  77 115 153 191 229 267 305 343 381 419 457 495 533 571 609 647 685 723 761 799 837 875 913 951  989
 R1   2   3   4   5   6   7   8   9  10  11  12  13  14  15  16  17  18  19  20  21  22  23  24  25  26   27
```

Appendix Table 2. Individual seedling characteristics and infection patterns of mycorrhizal fungi indigenou to the dyke in the mycorrhizal outplanting study.

CODE FOR DATA HEADINGS

ID	SEEDLING NUMBER
HT	APICAL HEIGHT (MM)
AP AC	APICAL ACTIVITY (1 - ACTIVE; 0 - INACTIVE)
NO BR	NUMBER OF BRANCHES
SH WT	SHOOT WEIGHT (MG)
RT WT	ROOT WEIGHT (MG)
WT COU	WEIGHT OF COUNTED ROOTS (MG)
UN-COU	WEIGHT OF UNCOUNTED ROOTS (MG)
ID	SEEDLING NUMBER
INF	NUMBER OF UNINFECTED SHORT ROOTS
INT	NUMBER OF INFECTED SHORT ROOTS WITH INTRODUCED SPECIES

INFECTED INDIGENOUS SPECIES 1 - 10 AS FOLLOWS
1-ESTRAIN
2-I-TYPE
3-UNK BASID
4- FUZZY ASCO
5-HYALINE BASID
6-RHIZO
7-HYALINE FLOCCOSE
8-UNKNOWN
9-UNK FLOCCOSE
10-CENOCOCCUM
TOTAL SHORT ROOTS PER MG ROOT AND PER SEEDLING OUTSIDE PLUG

```
  ID  HT AP  NO  SH  RT  WT UN-   UN-  INT    INFECTED INDIGENOUS SPECIES           TOTAL SHORT ROOTS
         AC  BR  WT  WT COU COU   INF         1   2   3   4   5   6   7   8   9  10  /MG    /SEEDLING
  86  65  1   4 898 485  55  98   300   0   0   0   0   0   0   0   0   0   0   0   5.5    834.5
  98  54  0   1 582 500  59   0   145   0   0   0   0   0   0   0   0   0   0   0   2.5    145.0
 100  74  0   0 533 620  65  65   300   0   0   0   0   0   0   0   0   0   0   0   4.6    600.0
 104  44  1   2 296 232  14   0    84   0   0   0   0   0   0   0   0   0   0   0   6.0     84.0
 114  64  1   3 856 566  37 174   300   0   0   0   0   0   0   0   0   0   0   0   8.1   1710.8
 958  65  1   4 764 765  39 176   300   0   0   0   0   0   0   0   0   0   0   0   7.7   1653.8
 962  75  1   1 613 657  18   0   116   0   0   0   0   0   0   0   0   0   0   0   6.4    116.0
 966  54  0   2 578 566  14   0   138   0   0   0   0   0   0   0   0   0   0   0   9.9    138.0
 972  34  0   0 171 184   0   0  99999999999999999999999999999999999999999999   0    .0       .0
 982  80  1   11173 829  35 106   300   0   0   0   0   0   0   0   0   0   0   0   8.6   1208.6
 268  45  1   1 843 429  20  26   212  14  14  53   7   0   0   0   0   0   0   0  15.0    690.0
 270  61  1   1 635 494  37  51   236   7  48   0   0   0   0   0   0   0   0   0   7.9    692.1
 274  63  0   1 894 397  10   0    26   0  31   0   0   0   0   0   0   0   0   0   5.7     57.0
 284  58  0   2 723 347  26   0   261   0   0   0   0   0   0   0   0   0   0   0  10.0    261.0
 302  52  0   1 603 358  39   0   312   0   0   0   0   0   0   0   0   0   0   0   8.0    312.0
 616  64  1   1 789 489  29  94   162   2   0   0   0 136   0   0   0   0   0   0  10.3   1272.4
 620  68  1   3 866 481  29  73   128  17  27   2   0   0 126   0   0   0   0   0  10.3   1055.2
 630  68  1   5 856 301  65  45   290  10   0   0   0   0   0   0   0   0   0   0   4.6    507.7
 636  65  1   1 913 466  42  84   272  28   0   0   0   0   0   0   0   0   0   0   7.1    900.0
 640  64  1   2 893 512  43 172   242  58   0   0   0   0   0   0   0   0   0   0   7.0   1500.0
 344  61  1   2 852 377  36   0    78  64   0   0   0   0   0   0   0   0   0   0   3.9    142.0
 348  55  1   2 561 458  29  99   139 156   0   0   0   0   0   5   0   0   0   0  10.3   1324.1
 356  52  1   2 729 289  40   0   197  19   0   0   0   0   0   0   0   0   0   0   5.4    216.0
 362  55  1   3 827 565  30   0    12 122   0   0   0   0   0   0   0   0   0   0   4.5    134.0
 374  60  1   91277 433  40  17   152 148   0   0   0   0   0   0   0   0   0   0   7.5    427.5
 812  35  0   1 185 470   0   0  99999999999999999999999999999999999999999999   0    .0       .0
 816  73  1   8 843 660  49 111   151 148   0   0   0   0   0   0   0   0   0   0   6.1    976.3
 820  50  1   9 742 548  40 113   198 102   0   0   0   0   0   0   0   0   0   0   7.5   1147.5
 824  59  1  141373 715  53 303   216  84   0   0   0   0   0   0   0   0   0   0   5.7   2015.1
 830  65  1   4 883 568  60  10   229  71   0   0   0   0   0   0   0   0   0   0   5.0    350.0
 420  76  1   6 623 607  77   0    72  84   0   0   0   0   0   0   0   0   0   0   2.0    156.0
 422  54  1   1 516 389  36  20    13 287   0   0   0   0   0   0   0   0   0   0   8.3    466.7
 440  82  1   31888 591 119 286    20 280   0   0   0   0   0   0   0   0   0   0   2.5   1021.0
 448  85  1   51282 628  49 293    69 231   0   0   0   0   0   0   0   0   0   0   6.1   2093.9
 450  74  1   31119 618  60 244   100 200   0   0   0   0   0   0   0   0   0   0   5.0   1520.0
 464  59  1   21146 581  65 136   175 125   0   0   0   0   0   0   0   0   0   0   4.6    927.7
 470  76  1   2 918 363  50   0    54 258   0   0   0   0   0   0   0   0   0   0   6.2    312.0
 472  58  1   2 830 781  67  63   103 197   0   0   0   0   0   0   0   0   0   0   4.5    582.1
 480  53  1   7 623 573  45 158    58 242   0   0   0   0   0   0   0   0   0   0   6.7   1353.3
 492  63  1   61106 703  39 326    13 287   0   0   0   0   0   0   0   0   0   0   7.7   2807.7
 382  48  1   6 340 291  13   0    91   6  38   0   0   0   0   0   4   0   0   0  10.7    139.0
 392  58  1   2 495 146  41   0   149   9 144   0   0   0   0   0   0   0   0   0   7.4    302.0
 404  49  1   4 425 390  30   0   248   8   0   0   0   0   0   0   0   0   0   0   8.5    256.0
 412  65  1   2 481 162  30   0   247  12   0   0   0   0   0   0   0   0   0   0   8.6    259.0
 416  59  1   2 751 623  40  86   260  40   0   0   0   0   0   0   0   0   0   0   7.5    945.0
 728  50  0   2 755 445  25 109   286   5   5   4   0   0   0   0   0   0   0   0  12.0   1608.0
 732  85  1   4 455 252  49  44   256  24  17   3   0   0   0   0   0   0   0   0   6.1    569.4
 744  53  1   1 362 462  25   0    83   0   0   0   0   0   0   0   0   0   0   0   3.3     83.0
 746  52  1   2 386 404  30  28   267  33   0   0   0   0   0   0   0   0   0   0  10.0    580.0
 750  53  0   1 414 324  38   0   195  19   0   0   0   0   0   0   0   0   0   0   5.6    214.0
 496  63   1    6 637 501  50 118      0 300   0   0   0   0   0   0   0   0   0   0   6.0  1008.0
 504  58   1    4 458 397  53 104     19 281   0   0   0   0   0   0   0   0   0   0   5.7   888.7
 508  64   1    1 512 349  41  62      0 300   0   0   0   0   0   0   0   0   0   0   7.3   753.7
 530  70   1    3 930 463  39 363      0 300   0   0   0   0   0   0   0   0   0   0   7.7  3092.3
 532  59   1    2 594 384  48  63      2 298   0   0   0   0   0   0   0   0   0   0   6.3   693.7
 846  48   1    2 546 440  39 136      5 295   0   0   0   0   0   0   0   0   0   0   7.7  1346.2
 856  72   1    3 570 355  40 154     99 201   0   0   0   0   0   0   0   0   0   0   7.5  1455.0
 860  50   1    2 414 433  51  26      0 300   0   0   0   0   0   0   0   0   0   0   5.9   452.9
 866  49   1    1 458 424  72  40     31 269   0   0   0   0   0   0   0   0   0   0   4.2   466.7
 872  65   1    1 799 334  55   0     47 196   0   0   0   0   0   0   0   0   0   0   4.4   243.0
 652  64   0    1 711 429  36  80    290   0   0   0   0   0   0  10   0   0   0   0   8.3   966.7
 656  62   1    21104 670  31 166    300   0   0   0   0   0   0   0   0   0   0   0 [illegible].7  1906.5
 668  56   0    31153 665  36 215    300   0   0   0   0   0   0   0   0   0   0   0   8.3  2091.7
 678  58   1    3 782 652  20 280    292   8   0  23   0   0   0   0   0   0   0   0  16.1  4845.0
 682  55   1    1 328 391  26  26    296   0   0   0   0   0   4   0   0   0   0   0  11.5   600.0
 996  45   0    2 451 498  48  11    300   0   0   0   0   0   0   0   0   0   0   0   6.3   368.7
 998  80   1    2 876 512  46 116    273  27   0   0   0   0   0   0   0   0   0   0   6.5  1056.5
1000  67   1    3 769 479  58   0    284  19   0   0   0   0   0   0   0   0   0   0   5.2   303.0
1012  74   0    4 943 492  31 100    299   1   0   0   0   0   0   0   0   0   0   0   9.7  1267.7
1024  62   1    21242 699  27 300    299   1   0   0   0   0   0   0   0   0   0   0  11.1  3633.3
 234  51   0    0 627 345  22   0    113   0 178   0   0   0   0   0   0   0   0   0  13.2   291.0
 246  62   1    1 726 471  81   0    234   0   0   0   0   0   0   0   0   5   0   0   3.0   239.0
 248  64   1    3 955 731  27  56    263   0  37   0   0   0   0   0   0   0   0   0  11.1   922.2
 262  70   1    1 746 447  31  49    295   0   0   0   0   0   5   0   0   0   0   0   9.7   774.2
 264  51   1    1 660 558  47  85    300   0   0   0   0   0   0   0   0   0   0   0   6.4   842.6
 770  52   1    1 593 363  50   9    290  20   0   0   0   0   0   0   0   0   0   0   6.2   365.8
 774  55   1    3 611 471  41  60    289   0   0   0   0   0  11   0   0   0   0   0   7.3   739.0
 786  47   1    1 803 711  51 119    282   0   0   0   0   0  18   0   0   0   0   0   5.9  1000.0
 794  49   1    3 756 567  57 149    297   0   0   3   0   0   0   0   0   0   0   0   5.3  1084.2
 796  52   1    1 816 538  40 108    298   1   0   0   0   0   0   2   0   0   0   0   7.5  1113.7
 536  57   0    1 651 554  54  56    138 162   0   0   0   0   0   0   0   0   0   0   5.6   611.1
 542  68   1    0 528 346  64   0     92  73   0   0   0   0   0   0   0   0   0   0   2.6   165.0
 562  57   1    2 878 512  42 271    112 188   0   0   0   0   0   0   0   0   0   0   7.1  2235.7
 568  82   1    1 452 423  52  67    236  64   0   0   0   0   0   0   0   0   0   0   5.8   686.5
 570  69   1    1 950 491  98   0    139  81   0   0   0   0   0   0   0   0   0   0   2.2   220.0
 574  60   1    1 687 499  69 282    123 160  17   0   0   0   0   0   0   0   0   0   4.3  1526.1
 582  72   1    41532 683  85 381    167 133   0   0   0   0   0   0   0   0   0   0   3.5  1644.7
 588  55   0    1 684 437  64  68    145 163   0   0   0   0   0   2   0   0   0   0   4.8   639.4
 592  62   1    21147 736  88 363    118 182   0   0   0   0   0   0   0   0   0   0   3.4  1537.5
 602  59   1    3 584 675  72  89    157 144   0   0   0   0   0   0   0   0   0   0   4.2   673.1
  52 175   1    31665 959  32 148    300   0   0   0   0   0   0   0   0   0   0   0   9.4  1687.5
  54 149   1    62447 919  58 425    300   0   0   0   0   0   0   0   0   0   0   0   5.2  2498.3
  58 128   1   1034501670  51 338    300   0   0   0   0   0   0   0   0   0   0   0   5.9  2288.2
  64 124   1    629491149  26 394    399   0   0   0   0   0   1   0   0   0   0   0  15.4  6461.5
  70 151   1    726371607  37 274    297   0   0   0   0   0   3   0   0   0   0   0   8.1  2521.6
 122 128   1    52462 966  59 408    292   0   8   0   0   0   0   0   0   0   0   0   5.1  2374.6
 128 129   1    51910 815  52 122    167   0 133   0   0   0   0   0   0   0   0   0   5.8  1003.8
 132 134   1    936261200  52 223    293   0   0   0   0   0   7   0   0   0   0   0   5.8  1586.5
 134 170   1    42436 884  46 230    300   0   0   0   0   0   0   0   0   0   0   0   6.5  1800.0
 146 126   1    52221 688  38 133    287   0   0   0   0   0   0  13   0   0   0   0   7.5  1350.0
 686  57   1    4 878 411  34 269    289   0   9   2   0   0   0   0   0   0   0   0   8.8  2673.5
 692  52   1    2 323 552  53   0    246   0   0   0   0   0   0   0   0   0   0   0   4.6   246.0
```

702	54	1	2	790	700	45	185	300	0	0	0	0	0	0	0	0	0	0	0	6.7	1533.3
706	55	1	7	798	640	47	107	300	0	0	0	0	0	0	0	0	0	0	0	6.4	983.0
712	57	1	2	695	584	28	68	300	0	0	0	0	0	0	0	0	0	0	0	10.7	1028.6
884	55	1	2	510	637	30	0	221	0	0	0	0	0	0	0	0	0	0	0	7.4	221.0
886	61	0	0	546	465	31	0	129	0	0	0	0	0	0	0	0	0	0	0	4.2	129.0
892	47	0	1	379	478	75	0	312	0	0	0	0	0	0	0	0	0	0	0	4.2	312.0
894	62	1	4	658	472	28	18	300	0	0	0	0	0	0	0	0	0	0	0	10.7	492.9
910	65	1	4	1002	547	53	93	300	0	0	0	0	0	0	0	0	0	0	0	5.7	826.4
2	69	1	7	1556	705	46	141	260	0	0	2	0	0	39	0	0	0	0	0	6.5	1223.6
10	54	1	3	549	434	32	0	229	0	0	0	0	0	0	0	0	0	0	0	7.2	229.0
20	49	1	1	765	449	32	22	300	0	0	0	0	0	0	0	0	0	0	0	9.4	506.2
32	69	1	4	887	669	63	121	292	0	8	0	0	0	0	0	0	0	0	0	4.8	876.2
34	48	1	2	703	659	48	179	293	0	2	0	0	0	0	5	0	0	0	0	6.3	1418.7
920	57	1	4	588	572	34	86	190	0	110	0	0	0	0	0	0	0	0	0	8.8	1058.8
928	62	1	4	1322	1105	57	148	345	0	4	0	0	0	0	1	0	0	0	0	6.1	1258.8
936	58	1	2	1132	632	37	66	300	0	0	0	0	0	0	0	0	0	0	0	8.1	835.1
938	45	1	1	689	380	30	0	175	0	0	0	0	0	0	0	0	0	0	0	5.8	175.0
942	47	0	5	389	405	43	0	179	0	0	0	0	0	0	0	0	0	0	0	4.2	179.0
154	48	0	0	573	448	45	13	300	0	0	0	0	0	0	0	0	0	0	0	6.7	386.7
170	49	0	2	1084	656	34	116	300	0	0	0	0	0	0	0	0	0	0	0	8.8	1323.5
180	67	1	1	1139	687	38	154	300	0	0	0	0	0	0	0	0	0	0	0	7.9	1515.8
182	67	0	1	654	476	36	14	300	0	0	0	0	0	0	0	0	0	0	0	8.3	416.7
188	62	1	8	1325	967	74	409	299	0	0	0	0	0	0	0	0	0	1	0	4.1	1958.1
308	56	1	2	499	293	20	0	184	0	5	0	0	0	0	0	0	0	0	0	9.4	189.0
320	62	1	3	697	497	32	47	300	0	0	0	0	0	0	0	0	0	0	0	9.4	740.6
326	73	0	1	1023	673	53	49	294	0	0	6	0	0	0	0	0	0	0	0	5.7	577.4
332	54	1	1	938	689	45	17	300	0	0	0	0	0	0	0	0	0	0	0	6.7	413.3
338	43	1	6	732	521	34	69	300	0	0	0	0	0	0	0	0	0	0	0	8.8	908.8
194	49	1	4	724	630	51	137	298	0	2	0	0	0	0	0	0	0	0	0	5.9	1105.9
196	60	1	2	723	530	22	72	286	0	14	0	0	0	0	0	0	0	0	0	13.6	1281.8
200	54	1	2	794	619	54	148	299	0	9	0	0	0	0	6	0	0	0	14	6.1	1227.0
222	76	1	1	1088	629	33	168	300	0	0	0	0	0	0	0	0	0	0	0	9.1	1827.3
226	74	1	5	911	711	37	114	294	0	0	6	0	0	0	0	0	0	0	0	8.1	1224.3

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PERCENT INFECTION BY INTRODUCED AND INDIGENOUS SPECIES (see code for species names)
                                                     INDIGENOUS SPECIES
 ID UNINFECTED INTRODUCED  1      2      3      4      5      6      7      8      9      10
  86   100.0      .0       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
  98   100.0      .0       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 100   100.0      .0       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 104   100.0      .0       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 114   100.0      .0       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 958   100.0      .0       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 962   100.0      .0       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 966   100.0      .0       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 982   100.0      .0       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 268    70.7     4.7      4.7   17.7    2.3     .0     .0     .0     .0     .0     .0     .0
 270    81.1     2.4     16.5     .0     .0     .0     .0     .0     .0     .0     .0     .0
 274    45.6      .0     54.4     .0     .0     .0     .0     .0     .0     .0     .0     .0
 284   100.0      .0       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 302   100.0      .0       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 616    54.0      .7       .0     .0     .0   45.3     .0     .0     .0     .0     .0     .0
 620    42.7     5.7      9.0     .7     .0     .0   42.0     .0     .0     .0     .0     .0
 630    96.7     3.3       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 636    90.7     9.3       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 640    80.7    19.3       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 344    54.9    45.1       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 348    46.3    52.0       .0     .0     .0     .0     .0    1.7     .0     .0     .0     .0
 356    91.2     8.8       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 362     9.0    91.0       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 374    50.7    49.3       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 816    50.5    49.5       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 820    66.0    34.0       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 824    72.0    28.0       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 830    76.3    23.7       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 420    46.2    53.8       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 422     4.3    95.7       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 440     6.7    93.3       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 448    23.0    77.0       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 450    33.3    66.7       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 464    58.3    41.7       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 470    17.3    82.7       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 472    34.3    65.7       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 480    19.3    80.7       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 492     4.3    95.7       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 382    65.5     4.3     27.3     .0     .0     .0     .0     .0     .0     .0     .0     .0
 392    49.3     3.0     47.7     .0     .0     .0     .0     .0    2.9     .0     .0     .0
 404    96.9     3.1       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 412    95.4     4.6       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 416    86.7    13.3       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 728    95.3     1.7      1.7    1.3     .0     .0     .0     .0     .0     .0     .0     .0
 732    85.3     8.0      5.7    1.0     .0     .0     .0     .0     .0     .0     .0     .0
 744   100.0      .0       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 746    89.0    11.0       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 750    91.1     8.9       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
 496      .0   100.0       .0     .0     .0     .0     .0     .0     .0     .0     .0     .0
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504	6.3	93.7	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
508	.0	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
530	.0	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
532	.7	99.3	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
846	1.7	98.3	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
856	33.0	67.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
860	.0	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
866	10.3	89.7	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
872	19.3	80.7	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
652	96.7	.0	.0	.0	.0	.0	.0	3.3	.0	.0	.0	.0
656	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
668	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
678	90.4	2.5	.0	7.1	.0	.0	.0	.0	.0	.0	.0	.0
682	98.7	.0	.0	.0	.0	.0	1.3	.0	.0	.0	.0	.0
996	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
998	91.0	9.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
1000	93.7	6.3	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
1012	99.7	.3	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
1024	99.7	.3	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
234	38.8	.0	61.2	.0	.0	.0	.0	.0	.0	.0	.0	.0
246	97.9	.0	.0	.0	.0	.0	.0	.0	.0	2.1	.0	.0
248	87.7	.0	12.3	.0	.0	.0	.0	.0	.0	.0	.0	.0
262	98.3	.0	.0	.0	.0	.0	1.7	.0	.0	.0	.0	.0
264	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
770	93.5	6.5	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
774	96.3	.0	.0	.0	.0	.0	3.7	.0	.0	.0	.0	.0
786	94.0	.0	.0	.0	.0	.0	6.0	.0	.0	.0	.0	.0
794	99.0	.0	.0	1.0	.0	.0	.0	.0	.0	.0	.0	.0
796	99.0	.3	.0	.0	.0	.0	.0	.7	.0	.0	.0	.0
536	46.0	54.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
542	55.8	44.2	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
562	37.3	62.7	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
568	78.7	21.3	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
570	63.2	36.8	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
574	41.0	53.3	5.7	.0	.0	.0	.0	.0	.0	.0	.0	.0
582	55.7	44.3	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
588	46.8	52.6	.0	.0	.0	.0	.0	.6	.0	.0	.0	.0
592	39.3	60.7	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
602	52.2	47.8	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
52	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
54	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
58	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
64	99.7	.0	.0	.0	.0	.0	.2	.0	.0	.0	.0	.0
70	99.0	.0	.0	.0	.0	.0	1.0	.0	.0	.0	.0	.0
122	97.3	.0	2.7	.0	.0	.0	.0	.0	.0	.0	.0	.0
128	55.7	.0	44.3	.0	.0	.0	.0	.0	.0	.0	.0	.0
132	97.7	.0	.0	.0	.0	.0	2.3	.0	.0	.0	.0	.0
134	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
146	95.7	.0	.0	.0	.0	.0	.0	4.3	.0	.0	.0	.0
686	96.3	.0	3.0	.7	.0	.0	.0	.0	.0	.0	.0	.0
692	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
702	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0

706	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
712	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
884	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
886	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
892	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
894	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
910	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
2	86.4	.0	.0	.7	.0	.0	13.0	.0	.0	.0	.0	.0
10	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
20	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
32	97.3	.0	2.7	.0	.0	.0	.0	.0	.0	.0	.0	.0
34	97.7	.0	.7	.0	.0	.0	.0	1.7	.0	.0	.0	.0
920	63.3	.0	36.7	.0	.0	.0	.0	.0	.0	.0	.0	.0
928	98.6	.0	1.1	.0	.0	.0	.0	.3	.0	.0	.0	.0
936	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
938	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
942	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
154	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
170	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
180	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
182	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
188	99.7	.0	.0	.0	.0	.0	.0	.0	.0	.0	.3	.0
308	97.4	.0	2.6	.0	.0	.0	.0	.0	.0	.0	.0	.0
320	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
326	98.0	.0	.0	2.0	.0	.0	.0	.0	.0	.0	.0	.0
332	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
338	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
194	99.3	.0	.7	.0	.0	.0	.0	.0	.0	.0	.0	.0
196	95.3	.0	4.7	.0	.0	.0	.0	.0	.0	.0	.0	.0
200	91.2	.0	2.7	.0	.0	.0	.0	1.8	.0	.0	.0	4.3
222	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
226	98.0	.0	.0	2.0	.0	.0	.0	.0	.0	.0	.0	.0

Appendix Table 3. Distinguishing features of jack pine mycorrhizae developing in muskeg peat under greenhouse conditions. Numbers conform to those in Tables 3 and 5, in Part 6.

1. I-type Ascomycete. Dichotomously branched once or twice, cream becoming rusty tawny with age, glabrous or bristling with hyaline cystidia. In plan view (500X) the mantle a textura epidermoidea (jig-saw puzzle-like), septa with associated Woronin bodies, cells up to 20 μm diameter. Cystidia hyaline, acute, 3 x 110-150 μm, wall slightly thickened to 0.8 μm, septate, base inflated, Woronin bodies at the septa. Growth in culture moderately rapid but sparse and with no aerial hyphae.

2. Tomentella sp. I. Dichotomously branched up to three times, dark brown to almost black, glabrous or occasionally with cystidia or a few extramatrical hyphae, hyphae 3-4 μm diameter, ochraceous, clamped, pigmented and smooth. In plan view the mantle a textura angularis, individual cells (5) 10-14 (20) μm diameter, surface smooth or furfuraceous with pigmented flakes deposited between cells. Individual cells easily seen at 25X. Cystidia generally absent; hyphoid, undifferentiated, tip obtuse, wall thin and lightly pigmented, 15-20 X 2-3 μm, septate with one simple septum. In culture colonies brown floccose, aerial hyphae 3-4 μm diameter, smooth or verruculose with encrustations, pale ochraceous, occasional cell contents turn green in KOH.

3. E-strain. Dichotomously branched once or twice, cream becoming sienna, rusty tawny to bag, nearly glabrous; extramatrical hyphae pale fawn, blister-like ornaments, 5.5 μm diameter excluding ornaments, 8.0 μm with ornaments. Mantle a textura epidermoidea - textura intricata, hyphal walls smooth, Woronin bodies present. Heavy intracellular infection or hyphae apparently restricted to Hartig net. Cultures typical of E-strain with stiff, frosted-appearing hyphae growing from the mycorrhizal root tips, initially hyaline, gradually becoming pale brown.

4. Yellow cystidia. Monopodial, greenish yellow (3B4) on tips, base brown over yellow (4A8) (like Pluteus lutescens),

sometimes only the meristematic region yellow, no extramatrical hyphae, cystidia difficult to see. Mantle a textura angularis, cells 6-15 μm diameter. Cystidia hyphoid, walls thin or thickened, up to 2 μm thick at the base, septate and clamped, 4 x 30-75 (105) μm, occasionally branched at right angle, tip obtuse and slightly inflated and occasionally with yellow resinous exudate on the tip. In culture, colony bright yellow.

5. Cenococcum geophilum. Black with stiff radiating hyphae. Hyphae 4-5.5 μm, smooth, walls .8-1 μm thick, septa thickening with age, Woronin bodies present at young septa. Cells of mantle nested.

6. Golden floccose. Densely floccose with yellow or golden brown hyphae. Hyphae smooth, 4 μm diameter with large hemisphaerical clamps. Mantle a textura intricata. Despite repeated attempts, the symbiont could not be cultured.

7. Rhizopogon-like I. Stout coralloid, floccose to felty with loose mycelial strands, white becoming vinaceous purple or nearly black with age. Microscopically the hyphae covered with plate - like crystals and lens of resinous exudate, septa simple. Crystalline material and resinous material hyaline or pale vinaceous, the colour intensifying when mounted in KOH. Infection usually in localized areas of the root. Readily cultured.

8. Rhizopogon II. Coralloid, bay, small amount of extramatrical hyphae, hyphae clamped; rhizomorphs dark, well developed. Hyphae with abundant crystals and vinaceous resin-like deposits. Growth good in culture.

9. T. angularis. Monpodial or occasionally dichotomous, pale cream, sparce extramatrical hyphae, hyphae 4-5 μm diameter, simple septate, hyaline, smooth. Mantle composed of blocky elements forming a textura angularis, cells 8-14 μm diameter, Woronin bodies present. Growth in culture unknown.

10. Tibiiform cystidia. Cream, appearing finely hairy due to cystidia. Mantle a textura angularis, cells 10-14 μm diameter. Cystidia tibiiform, 80-110 μm long, arising from narrow hyphae on the surface, all thin walled, smooth, tip 6-10 μm diameter, base 3-4 μm

diameter with a simple septum. Woronin bodies absent. Not obtained in culture.

11. Black asco. Jet black with abundant black hyphae radiating out as with Cenococcum; hyphae stiff, infrequently branched, densely ornamented with blister-like warts, 1.5 μm high, hyphae 4-4.7 μm diameter exclusive of ornaments, 6.3-7.1 μm with ornaments. Not obtained in culture.

12. Hyaline basidio. Dichotomous, cream, unpigmented, extramatrical mycelium sparse or not apparent and the mycorrhizae appearing glabrous. Hyphae hyaline, smooth, 4 μm diameter, clamped, mantle a compact textura intricata, thin. The mycorrhizae are undistinguished and culturing was required to confirm identification. In culture on MMN, growth rapid, aerial hyphae lacking, colony hyaline, spreading, submerged hyphae 4-6 μm diameter, smooth, clamped, remaining separate or forming small aggregations giving the colony a spotty appearance. Hyphae in aggregations moniliform 10-15 (26) μm diameter, walls 1-1.5 μm thick. All colonies turn pale yellow with the application of KOH or NaOH and this character separates this taxa from all the other hyaline, clamped species.

13. Coarse asco. Short roots appearing uninfected except for the opaque nature or becoming dichotomous, no extramatrical mycelium or very sparse, 4-6 μm diameter, walls slightly thickened, hyaline or tinted brown non-ornamented Woronin bodies conspicuous at the septa. Microscopically the mantle thin as with the E-strain, often discontinuous cells 6-12 (22) μm diameter, suggestive of a textura globulosa or a textura intricata with inflated cells or textura intricata-textura epidermoidea. Intracellular infections absent. Slight growth from washed tips in pure culture but no growth after transfer.

14. White floccose. Monopodial to dichotomous, stout, floccose; hyphae 2.5 μm diameter, hyaline, thin-walled, clamped.

15. Unknown affinity. Material too scanty to characterize.

16. Unknown affinity. Dichotomously branched 1-2X, long elements, cream, consistently with sparse extramatrical hyphae; hyphae hyaline, 2.5 μm diameter, smooth, simple septate.

17. Unknown affinity. Dichotomously branched once or twice, elements stout, cream becoming pale brown with age, completely glabrous. Mantle dense and compact, a textura epidermoidea, cells 2.5-4.5 µm diameter; hyphae simple septate, no Woronin bodies seen. No growth on MMN$^+$ from washed tips.

18. Basidiomycete. Unbranched, floccose, tinted yellow; hyphae 2.5 µm diameter, clamped, smooth or verruculose. Suggests *Amphinema* but culturing required to confirm.

19. Unknown affinity. Floccose, cream, robust, hyphae 2 µm diameter, smooth, simple septate, Woronin bodies lacking. No growth on benomyl-MMN after washing tips.

20. *Tomentella*-like. Dark brown, glabrous and very similar to *Tomentella*. Mantle a textura angularis, cells up to 10 µm diameter, with discontinuous bits of pigments between cells which project out and at 25X give a rough appearance, i.e. flake-like deposits. In culture (R-2350) growth slow with abundant long erect fascicles of hyphae, hyaline becoming dingy brown, rarely cells globose and 10 µm diameter. Hyphae 3-4 µm diameter, smooth, hyaline clamped.

21. *Tomentella* II. Dichotomously branched up to 3 times, snuff brown, bay becoming fuscous black, glabrous or with a dense proliferation of flexuous cystidia, occasional hyphae radiating out, 3 µm diameter, pale yellow brown, finely roughened. Mantle a textura epidermoidea, cells 3-6 (10) µm diameter, with or without flaky incrustations. Cystidia 2-2.5 x 200-250 (340) µm acute, walls hyaline to ochraceous, clamped at the base, nearly occluded, aseptate or with one simple septum; arising from a low turf of much branched hyphae 2.5-5.5 µm diameter. In culture deep floccose, fawn, reverse dark brown, aerial hyphae 4 µm diameter, smooth or finely encrusted, hyaline to ochraceous, in 10% KOH a few encrusted areas flash through violet then turn green and slowly dissolve. On PDA small areas of hyphal walls are green in KOH.

22. Ascomycete. Blackish, hyphae brown, simple septate with Woronin bodies, 6 µm diameter; mantle a textura intricata, cells 4-8 µm diameter.

23. Unknown affinity. Sparse mycelium.

24. Basidiomycete. Floccose, white, monopodial. Hyphae 3-4 µm diameter, smooth, clamped. No growth from surface sterilized tips.

25. Ascomycete. Black and Cenococcum-like but dark radiating hyphae not as stiff, hyphae 4 µm diameter, smooth, simple, obvious Woronin bodies; mantle cells up to 20 µm diameter.

26. White floccose. Pure white, floccose, white mycelial strands. Hyphae smooth, 4 µm diameter, large clamps present. Growth in pure culture.

27. Basidiomycete. Insufficient material to determine features.

28. Unknown affinity. Insufficient material to determine characteristics.

29. Basidiomycete. Elements slender, reddish brown, no external mycelium. Hyphae 2.5-4 µm diameter, clamped, mantle thin, discontinuous.

30. White floccose. Dense floccose, hyphae 4 µm diameter, hyaline, smooth, clamped.

31. Dense floccose. Densely floccose with hyaline mycelium, surface pale cream; hyphae 2.5-3.5 µm, smooth, clamped.

Appendix Table 4. Operational levels of fertilizers used in producing container-grown seedlings.

Reference	Tree species	Planting mix and container size	Fertilizer formulation	Days between applications	Rate of Application (mg N:P:K/l)
Carlson (1979)	Jack pine	Peat, 47 cc	229:29:154	7	229:29:154
			44:101:50	14	44:101:50
B. Mattis, pers. comm.	Jack pine	P-V (2:1), 47 cc	20:20:20	7	200:80:170
Tinus & McDonald (1979)	Ponderosa pine	?, 164 cc	-	<7	223:27:155
B. Fessenden, pers. comm.	Jack pine	Peat, 160 cc	10:52:10	2	40:91:33
			20:20:20	2	100:44:83
			10:52:10	7	40:91:30
Kropp (1982)	Hemlock	P-V (1:1), 98 cc	10:52:16	3	62:143:83
			20:20:20	3	100:44:83
			0:52:34	3	0:143:176
			10:52:16	3	62:143:83
Arnott (1983)	Douglas-fir hemlock	?	10:52:10	3	62:143:52
			28:14:14	3	87:19:36
			NH_4NO_3	10	108:0:0

Appendix Table 5. Fertilizer levels used in producing mycorrhizal containerized seedlings.

Reference	Tree species	Planting mix and container size	Fertilizer formulation	Days between applications	Rate of Application
Molina (1979)	Douglas-fir, Lodgepole pines	P-V (1:1), 65 cc	20:19:18	7	12 g/m^2
Molina (1980)	Conifers	P-V (1:1), 165 cc	20:19:18	14	6 g/m^2
Shaw & Molina (1980)	Sitka spruce	P-V (1:1), 65 cc	20:20:20	3	200 mg/l
Ruehle (1980a)	Oak			21	500 mg N/l
Ruehle (1980b)	Loblolly pine	Bark-V (9:1)	23:19:17	21	575 mg N/l
Ruehle *et al.* (1981)	Shortleaf pine	Bark	20:19:18	21	575 mg N/l
Ruehle *et al.* (1981)	Shortleaf pine	Bark	20:19:18	7	575 mg N/l
Riffle & Tinus (1982)	Ponderosa and scots pine	P-V (1:1), 410 cc	Low N	<7	20:60:155 mg NPK/l
Kropp (1982)	Hemlock	P-V (1:1), 98 cc	20:20:20	3	100:44:83
Marx *et al.* (1982)	Sand and slash pines	Bark	23:19:17	21	30 ml/seedling of 575 mg N/l

(cont'd)

Appendix Table 5. (cont'd)

Reference	Tree species	Planting mix and container size	Fertilizer formulation	Days between applications	Rate of Application
Marx *et al.* (1982)	Virginia pine	P-V (3:1), 40 cc	15:4:22	2 wk	15:2:35 mg NPK/1
			150:42:122	7	150:18:101 mg NPK/1
			15:100:33	7 wk	15:44:27 mg NPK/1
			0:0:83	9 wk	0:0:69 mg NPK/1
Marx *et al.* (1982)	Ponderosa and Scots pine	P-V (1:1), 410 cc	20:31:155	<7	20:31:155 mg NPK/1
Marx *et al.* (1982)	Short and longleaf pines	P-V (1:1), 75 ml	20:19:18	5 and 10 wk	15 ml/cell of 575 mg N/1
Marx *et al.* (1982)	Ponderosa, Scots and Austrial pine	P-V (1:1), 495 cc	7:6:19	11	15 ml/cell of 32:12:72 mg NPK/1
Marx *et al.* (1982)	Oak	P-V (1:1), 410 cc	High N	10	223:27:155 mg NPK/1
Marx *et al.* (1982)	Douglas-fir and hemlock	P-V (1:1), 65 cc	20:19:18	14	15 ml/cell of 120:50:90 mg NPK/1
Marx *et al.* (1982)	Jack pine	P-V (3:2), 40 cc	20:20:20 + 540 mg/1 NH_4NO_3	4x/wk	12 ml of 529:194:365 mg NPK/1

(cont'd)

Appendix Table 5. (cont'd)

Reference	Tree species	Planting mix and container size	Fertilizer formulation	Days between applications	Rate of Application
Marx et al. (1982)	Jack pine	P-V (3:2), 40 cc	20:20:20 + 540 mg/l NH_4NO_3	2x/wk	12 ml of 529:194:365 mg NPK/l
Marx et al. (1982)	Loblolly pine	P-V (1:1) ?	20:20:20 + 540 mg/l NH_4NO_3	2 and 8 wk	25 ml/cell; 200 mg N, 20 mg P
Graham & Lindemann (1981)	Douglas-fir	P-V-sand (1:1:1) 150 cc	-	7	94:66:82 mg NPK/l
Cline & Reid (1982)	Ponderosa and lodgepole pine	P-V (1:2:5), 625 cc		14	55:15:160 mg NPK/l
Danielson et al.	Jack pine	P-V (1:1), 165 cc	15:15:18	2	15:7:15 mg NPK/l
Danielson et al.	Jack pine	P-V (1:1), 165 cc	15:15:18	2	30:12:30 mg NPK/l
Danielson et al.	Jack pine	P-V (1:1), 165 cc	15:15:18	2	60:26:60 mg NPK/l
Danielson et al.	Jack pine	P-V (1:1), 165 cc	15:15:18	2	120:53:120 mg NPK/l

10.6 APPENDIX LITERATURE CITED

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